The potential of *Cleobora mellyi* (southern ladybird) as a biological control agent of the invasive tomato-potato psyllid (*Bactericera cockerelli*) on potatoes

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by

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Invasive species cause large annual economic losses worldwide, including the New Zealand agricultural and horticultural sectors, and threaten our native and endemic species. Insects are some of the most damaging invasive species in New Zealand horticultural production, causing crop losses either directly by plant damage, or indirectly by vectoring disease. The effective management of these insects is critical to maintaining viable industries, but must be done in such a way as to limit, where possible, environmental harm.

In this thesis I focussed on the biological control of the invasive insect tomato-potato psyllid (*Bactericera cockerelli*), an exotic pest of solanaceous crops from Central America. The biological control agent I investigated was the southern ladybird (*Cleobora mellyi*). The tomato-potato psyllid was first detected in New Zealand in 2006, rapidly dispersing around the country. This pest also causes a physiological condition known as psyllid yellows, and vectors a bacterium (*Candidatus* Liberibacter solanacearum syn. psyllaurous) that causes zebra chip disease in potato plants. The psyllid, psyllid yellows, and zebra chip disease combined are estimated to have cost the potato industry NZ $120 million from 2006-2011.

The southern ladybird was first introduced to New Zealand in the 1970s as a biological control agent for the eucalypt tortoise beetle (*Paropsis charybdis*), with further recent releases. Laboratory based consumption bioassays and behaviour analysis indicated that the southern ladybird had potential as a biological control agent, work which is developed furthered in this thesis.

Under glasshouse conditions, I investigated two main objectives for my first experimental chapter: 1) if the southern ladybird could reduce tomato-potato psyllid densities on potato plants; 2) if there was a trophic cascade caused by top-down control resulting in differences in

tuber number, weight, and dry matter. The results showed that over a 10 week sampling period, the southern ladybird significantly reduced TPP densities. Where there was only the psyllid, and no ladybird, no tubers were formed, while where there was the ladybird and psyllid, significantly smaller and fewer tubers were produced than the control and ladybird only.

To test for prey choice, I conducted a laboratory bioassay using small experimental arenas. Both adult and larval ladybirds were trialled using the same methodology. A single ladybird in each arena was presented with a choice between 10 psyllid nymphs and either 10 green peach aphids, potato aphids, or greenhouse whitefly nymphs. Neither the adult nor larvae of the southern ladybird showed a preference for the psyllid over either aphid species, but did show an aversion to the whitefly as prey.

I investigated the longevity of unmated adult southern ladybirds over a 12-week period using four treatments: 1) water only; 2) a floral resource (flowering buckwheat); 3) 10 psyllids/day; 4) flowering buckwheat + 10 psyllids/day. The unfed ladybirds showed 100% mortality within a two week timeframe. The ladybirds provided with only 10 psyllids/day, and those provided with only buckwheat survived significantly longer than those unfed. Ladybirds fed the combination of psyllids and buckwheat survived significantly longer than those fed only psyllids and the control.

The southern ladybird showed some potential as a biological control agent of the tomato-potato psyllid. Further research, such as testing for intraguild predation, and replicating the results in this thesis in semi-field experiments is required to determine if full-scale field releases of this predator as a biological control agent should progress.

Keywords: biological control, tomato-potato psyllid, southern ladybird, *Bactericera cockerelli*, *Cleobora mellyi*, psyllid yellows, zebra chip, potato, invasive species management, floral resources.

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Introduction

1.1 Overview

Invasive species pose a severe threat to natural and modified ecological habitats worldwide (Lockwood et al. 2007). New Zealand is no exception, with many threats to our native and endemic ecosystem as well as the agricultural and horticultural sectors (Clout and Lowe 2000, Goldson et al. 2005).These, and other pest species already cause substantial annual economic losses in these agricultural and horticultural sectors, estimated between $800 million and $2.13 billion annually (Goldson et al. 2005, Giera and Bell 2009). Invasive species are comprise a variety of taxa, including fungi, plants, vertebrates, and invertebrates (Clout and Lowe 2000).

There are at least 2200 different species of introduced invertebrate species in New Zealand (Ministry for the Environment 1997). Increasing levels of international trade and tourism have the potential to increase invertebrate incursions (Goldson et al. 2005). Global climate change may also make New Zealand climatically suitable for a number of other invasive invertebrates (Clout and Lowe 2000).

New Zealand's strict biosecurity protocols are aimed at pre-empting and preventing such incursions of invasive organisms. Biosecurity New Zealand screen passengers and luggage arriving at airports using trained detection dogs and X-ray machines (Goldson et al. 2005), also searching a selection of shipping containers (Goldson et al. 2005). One study revealed 6.1% of loaded, and 1.6% of unloaded containers searched contained live, regulated organisms (Ministry of Agriculture and Forestry 2003). Inevitably, some new organisms make it to New Zealand (e.g. Tomlinson 1973), many being difficult to detect (Kriticos et al. 2005). Only a fraction of the organisms transported will become established, spread, and have an impact (Goldson et al. 2005). Sometimes eradication is pursued, as with the painted apple moth (*Teia anartoides* Walker) (Suckling et al. 2007) but this is often proves difficult, uneconomical, or both (Clout and Veitch 2002).

Biological control is often used as a tool to help manage invasive insects in New Zealand's agriculture and horticulture industries of New Zealand (see Goldson et al. 2005). It has proven a useful management tool for pest and invasive insects in different circumstances in New Zealand. Examples where biological control has been beneficial include the management of

clover root weevil (*Sitona Lepidus* Gyllenhal) control by the parasitoid *Microctonus aethiopoides* Loan (Kean and Barlow 2000), the lucerne weevil (*Si. discoideus* Gyllenhal) by *M. aethiopoides* (Barlow and Goldson 1993), and leafrollers (Lepidoptera: Tortricidae) by *Dolichogenidea tasmanica* Cameron in vineyards (Stephens et al. 1998).

1.1.1 Psyllids

Psyllids (Psylloidea), frequently referred to as jumping plant lice, are small phloem-feeding hemipterans usually restricted to dicotyledonous plants, with most species utilising only a limited range of host plants (Eastop 1973). Along with aphids, whitefly and scale insects, they are in the suborder Sternorrhyncha (Grimaldi and Engel 2005). Psyllids are an ancient lineage, arising in the Permian (Hodkinson 1980), with an estimated 2500 described species distributed worldwide (Burckhardt and Lauterer 1997). Many economically important plants such as *Citrus* spp. and those in the family Solanaceae are vulnerable to psyllid damage (e.g., Burckhardt et al. 1999, Halbert & Manjunath 2004, Munyaneza 2010). Considered important agricultural pests worldwide (Munyaneza 2010), psyllids damage plants through two principal mechanisms; directly feeding on phloem sap, and/or vectoring plant pathogens (Halbert & Manjunath 2004, Munyaneza 2010).

The importance of psyllids' ability to vector plant diseases has been recently recognised in bothannual and perennial crops (Munyaneza 2010). '*Candidatus* Liberibacter' is a bacterial and plant pathogen vectored by at least five psyllid species (*Diaphorina citri* Kuwayama, *Trioza erytreae* Del Guercio, *T. apicalis* Förster, *Bactericera cockerelli* Sulc., *B. trigonica* Hodkinson) (Munyaneza 2010, Alfaro-Fernández et al. 2012). This particular bacterium type is associated with a range of historically - and newly recognised, economically important diseases in a range of different plant families and crop types, including potatoes (Bové 2006, Munyaneza 2010).

1.1.2 Potato industry profile

Potatoes are grown on approximately 10,670 hectares of land in New Zealand, with the total value of the industry estimated to be $382 million per year (Potatoes NZ 2011). A large proportion of the industry's value comes from exports. Most of this comprises frozen products, as fresh potatoes are banned from many international markets for phytosanitary reasons (Potatoes NZ 2011). There are three categories of potato production in New Zealand 1) grown for the table, 2) processing into potato products, 3) and seed potatoes (Potatoes NZ 2011).

The main potato growing regions are located predominantly in the North Island (Pukekohe, Hawkes Bay, Manawatu) and Canterbury in the South Island (Potatoes NZ 2011). There are approximately 50 cultivars of potato grown around New Zealand, but only a selection are used commercially (Potatoes NZ 2011). The main commercial cultivars grown include Russett Burbank, Innovator, Rua, Nadine, Agria, Moonlight, Desiree, Ilam Hardy and Red Rascal (Potatoes NZ 2011).

1.2 Study species

The tomato-potato psyllid, (TPP) *Bactericera cockerelli* Sulc. (Hemiptera: Triozidae) was originally placed in the *Trioza* genus in 1909, before being revised to the *Paratrioza* genus and revised again to the *Bactericera* genus (Abdullah 2008). There are at least 121 different species in this *Bactericera* genus (Burckhardt and Lauterer 1997). TPP is native to the Texas and Mexico area, where it is considered a pest species, that causes substantial losses in tomato crops (Liu et al. 2006). It has become an invasive pest species in the southern, central, and western United States of America; including, but not limited to the states of: Arizona, New Mexico, Texas, Nebraska, South and North Dakota, Minnesota, California, Washington, and Oregon (Al-Jabr 1999, Liu et al. 2006). It can also be found periodically in the Canadian provinces of Alberta, Saskatchewan, and British Columbia (Wallis 1955, Al-Jabr 1999) and is an invasive pest species in Honduras and Guatemala (Secor et al. 2009, Munyaneza 2010).

TPP was first detected in New Zealand in May 2006 (Gill 2006, Teulon et al. 2009, Thomas et al. 2011), and has since spread rapidly throughout the country (Teulon et al. 2009, Thomas et al. 2011). As a consequence of this rapid spread, an eradication programme was not pursued (Teulon et al. 2009). The entry pathway or transport vector for TPP into New Zealand is unclear, although genetic evidence suggests the incursion originated from the western USA (Thomas et al. 2011). Thomas et al. (2011) suggests that the arrival of TPP is unlikely to have occurred from nursery stock or fresh produce due to New Zealand's strict biosecurity protocols on imported goods. Natural dispersal is unlikely due to unsuitable weather patterns for TPP's self-introduction to New Zealand. Therefore, intentional, illegal introduction cannot be excluded (Thomas et al. 2011).

Liu et al. (2006) and Liu &Trumble (2007) have reported two biotypes of TPP in the USA, a native and invasive type. Liu and Trumble (2007) found the native biotype has a higher growth index and survivorship, with a lower developmental time than the invasive biotype. Testing for insecticidal resistance found the invasive biotype was resistant to the two common insecticides (imidacloprid and spinosad), while the native biotype showed no resistance. Liu

and Trumble's (2007) findings suggest that the invasive ability of TPP in the USA is related to its insecticide resistance, rather than a new biological or ecological trait. There is evidence that both biotypes are present in New Zealand (Berry et al. 2009).

1.2.1 Biology

Like all insects in the order Hemiptera, TPP is hemimetabolic, undergoing incomplete metamorphosis. Hemimetabolic insects have no pupal stage, so there are three distinct life-stages of egg, nymph, and adult. TPP develops through five nymph instars (Al-Jabr 1999, Teulon et al. 2009) before reaching sexually maturity. TPP eggs are golden coloured, less than 0.2 mm long and attached to the host plant on short stalks (Teulon et al. 2009). The nymphs are small (<3 mm), green, and dorso-ventrally flattened (Fig. 1a), while adults are similar in size but cicada-like-in-appearance (Fig. 1b) (Al-Jabr 1999, Teulon et al. 2009).

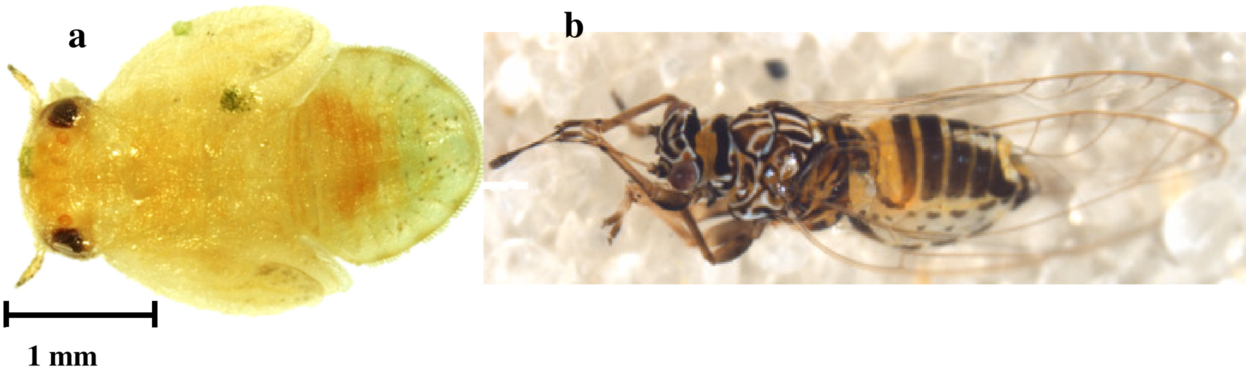


Figure 1. A fifth instar TPP nymph with scale bar (a), and an adult (b). Images courtesy Marie Macdonald

TPP disperse annually. How this is done is not well-understood in either the USA or New Zealand, though is known to be greatly aided by air currents (Knowlton and Janes 1931), with records from Mexico of TPP adults being found at altitudes of 1200 m (Glick 1939).

Although TPP is most commonly associated with the plant family Solanaceae, it has also been recorded on a number of other plant families in the USA (Al-Jabr 1999). These include Amaranthaceace, Asclepiadaceae, Asteraceae, Brassicaceae, Chenopodiaceae, Convolvulaceae, Fabaceae, Lamiaceae, Lycophyllaceae, Malvaceae, Menthaceae, Pinaceae, Poaceae, Polygonaceae, Ranunculaceae, Rosaceae, Salicaceae, Scrophulariaceae, Violaceae

and Zygophyllaceae (Pletsch 1947, Wallis 1955). It has been recorded reproducing only on plants in three of these families; Solanaceae, Convolvulaceae and Lamiaceae (Knowlton and Thomas 1934, Martin 2008).

In New Zealand, TPP have been found to successfully breed on important crops in the Solanaceae family, on confamilial weeds, and one native plant species (Martin 2008). TPP has affected commercial crops in New Zealand, mainly the potato (*Solanum tuberosum*), tomato (*So. lycopersicum*), capsicum (*Capsicum* spp.) and tamarillo (*So. betaceum*) industries (Teulon et al. 2009, Thomas et al. 2011). TPP can breed on the culturally important plants such as kumara (*Ipomoea batatas*) and poroporo (*S. aviculare*) (Martin 2008). However, not all hosts are equally suitable for TPP. Kumara, poroporo, *Datura stramonium*, and *Nicandra physalodes* are poor hosts which support only low densities of nymphs (Martin 2008).

The life-cycle of TPP within New Zealand is not fully understood, but is assumed to be similar to that in North America. On outdoor potato crops in North America, TPP usually have between four-seven generations per year (Abdullah 2008). The longevity and fecundity of TPP appears to be highly crop context specific, with a range of values for these life-history traits described (Liu and Trumble 2007, Abdullah 2008). Adult longevity has been recorded between 16-97 days (Butler and Trumble 2012 and references within). The optimal development for TPP occurs in the 15.5-32.2 °C temperature range, with a lower development threshold of 7 °C (Abdullah 2008). However, temperatures above 30°C can be lethal in some instances (Abdullah 2008). In optimal temperatures, the TPP can complete its life-cycle within 25-33 days (Abdullah 2008). The life-cycle biology is supported by laboratory data from New Zealand (Tran et al. 2012). Female fecundity values have been described between 147-399 eggs laid in a lifetime depending on the particular host plant and whether it is a field or laboratory setting (Knowlton and Janes 1931, Abdullah 2008, Yang et al. 2010a).

There is a 1:1 sex ratio of males to females when the nymphs hatch (Knowlton and Janes 1931). Research has demonstrated that male TPP are attracted to female TPP odorants. Guédot et al. (2010) found that TPP males were attracted to live females as well as female odorants extracted using solvents. Males were attracted to odorants from TPP males, but given a choice, were preferentially attracted to females. Females avoided other live females and solvent-extracted female odorants, as well as those odorants from live and extracted males.

1.3 Why is TPP a problem?

1.3.1 Psyllid sugars

Nymphs and adults excrete a white granular powder known as psyllid sugars (Compere 1916), a key indicator of TPP presence and feeding. The psyllid sugars can facilitate the growth of black sooty type fungi on the infested plant, reducing photosynthetic rates (Resh and Cardé 2009).

1.3.2 Psyllid yellows

Psyllid yellows refers to a physiological plant condition induced by TPP. Although no longer considered to be the most important aspect of TPP as a pest, psyllid yellows was originally a key concern in the event of a TPP infestation. The condition results in yield reductions in potatoes, with smaller, misshapen, less marketable tubers that have a rough skin (Eyer 1937, Schaal 1938, Cranshaw 1994). These tubers often have a disrupted dormancy period, sprout prematurely, or produce chain tubers (Cranshaw 1994).

The symptoms of psyllid yellows include: a yellowing or purpling of potato leaflets and shoots, stunted shoots, aerial tubers forming, increased number of axillary buds, early plant senescence, and shortened and thickened internodes (Eyer and Crawford 1933, Eyer 1937, Munyaneza et al. 2007a, Sengoda et al. 2010). Removing TPP from plants with psyllid yellows symptoms can lead to the plants full recovery (Eyer 1937, Sengoda et al. 2010).

The exact cause of psyllid yellows is unclear (Sengoda et al. 2010). Carter (1950 cited in Al-Jabr 1990, Cranshaw 1994) asserts “chlorotic toxaemia” as the cause –the result of toxins injected during TPP feeding. Carter (1950 cited in Al-Jabr 1990) also found that this condition could only be induced by the TPP nymphs, and not the adults. It has been postulated that the cause of psyllid yellows is either a yet-to-be-detected pathogen (Whitney Cranshaw, pers. comm. Colorado State University, 2012), or undetectably low levels of the bacterium responsible for zebra chip disease (Andrew Pitman, pers. comm. Plant & Food Research, 2012).The onset of psyllid yellows is not consistent, with observations of high nymph densities not inducing the condition (Cranshaw 1994).

Eyer (1937) documented the physiological and microscopic changes when TPP feed on leaves. TPP feeding resulted in increased protein breakdown, raised sucrose levels, lowered nitrogen and reduced starch levels. Carbohydrate metabolism was also affected by feeding injury. The rupturing of plant cells during TPP feeding caused smaller, lighter-coloured chloroplasts; suggesting that heavily damaged tissues had decreased chlorophyll and carotene

content. Eyer and Crawford (1933) found that the principal feeding site of TPP lends itself to the rapid translocation of anything injected by its feeding. This means TPP saliva and its contents can be systemically moved around the plant.

1.3.3 Zebra chip

The most significant problem caused by TPP is its vectoring of a plant pathogen (*Candidatus* Liberibacter solanacearum syn. psyllaurous) (Lso), purportedly responsible for the zebra chip disease in potatoes (Munyaneza et al. 2007b, Munyaneza 2010). The Lso bacterium, associated with zebra chip, has only recently been discovered in 2008 (Hansen et al. 2008, Liefting et al. 2009a, Liefting et al. 2009b). Genetic analysis shows there are multiple haplotypes of Lso, suggesting a long-standing separation with little gene flow between Lso populations (Nelson et al. 2011). Munyaneza (2010) reported that acquisition or transmission of a pathogen by a single TPP to and from the host plant takes approximately two hours. It takes approximately three weeks for zebra chip disease symptoms to develop in healthy plant foliage upon exposure to infected TPP (Munyaneza 2010). Evidence suggests this bacterium is capable of manipulating plant defence signalling and the defensive response, benefitting both the bacterium and TPP (Casteel et al. 2012). The plant pathogen also infects other crops in the Solanaceae plant family such as tomato, capsicum, eggplant and tamarillo (Liefting et al. 2009b).

The Lso pathogen was first recorded in 1994 in Mexico (Munyaneza et al. 2007b), and in Texas, USA in 2000 (Secor et al. 2009, Munyaneza 2010). It has since been found in California, Colorado, Kansas, Nebraska, Nevada, New Mexico, Washington, and Oregon (Secor et al. 2009, Crosslin et al. 2012) and in Central America in Honduras and Guatemala (Secor et al. 2009, Munyaneza 2010).

Zebra chip and psyllid yellows have very similar above ground symptoms (Munyaneza et al. 2007a, Sengoda et al. 2010), the key difference is subterranean, and undetectable without inspecting the tubers. The tuber symptoms for zebra chip are characterised by collapsed stolons, a browning of vascular tissue in the tubers and brown streaking of the medullary ray tissue (Miles et al. 2010, Munyaneza 2010) (Fig. 2). If infected potatoes are fried, these symptoms become much more pronounced, making them unacceptable for commercial use - tubers show dark spots, streaking, and stripes, (Munyaneza et al. 2007a, Munyaneza et al. 2008, Secor et al. 2009, Miles et al. 2010). In New Zealand, these ‘stripes’ can take more of a bruised appearance (Andrew Pitman, pers. comm. Plant & Food Research, 2011). Recent

work has shown that the cause of the tubers' symptoms are caused by cell death induced by the bacterium (Miles et al. 2010).

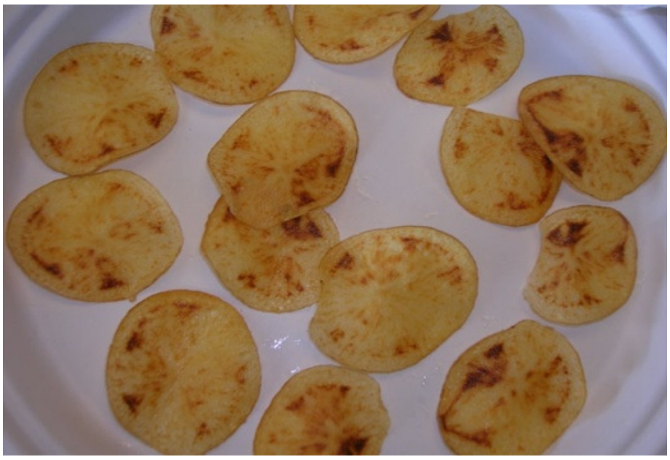


Figure 2. The typical symptoms of zebra chip in fried potatoes (Sourced: http://amarillo.tamu.edu/files/2010/12/Zebrachipthumbnail.jpg)

Infected seed tubers are significantly less likely to sprout than uninfected tubers (Henne et al. 2010). If infected tubers do sprout, only hair sprouts may be produced, and the plants have significantly reduced survival compared to uninfected plants (Henne et al. 2010). Pitman et al. (2011) found a tuber infected with Lso can pass the bacterium on through its foliage, and into progeny tubers. A large proportion of infected tubers produced asymptomatic foliage; however those with asymptomatic foliage usually produced daughter tubers free of the bacterium. Only two of the 62 infected tubers in their study showed the symptomatic signs in their foliage; these failed to produce daughter tubers. They concluded that asymptomatic tubers could have an important role in spreading zebra chip disease through New Zealand, by providing a source of acquisition for TPP and infected seed tubers being moved around the country. However, where zebra chip is already present, plants growing from infected tubers are unlikely to have a major contribution to zebra chip occurrence and spread (Henne et al. 2010).

Until the 2004-2006 growing seasons, zebra chip was mostly considered to be economically unimportant, until it began creating widespread, large-scale losses in Central America and south-western USA (Munyaneza et al. 2007a, Munyaneza et al. 2008). The consequences of zebra chip are reductions in the yield of potatoes, leading to losses of millions of dollars annually in the affected parts of the United States (Munyaneza et al. 2008). In New Zealand, TPP and zebra chip is estimated to have cost the potato industry $120 million since its discovery in 2006 (Plant & Food Research 2011).

In New Zealand, the disease can cause up to a 60% reduction in yield, and tubers may be of lower quality with less dry matter than uninfected crops (Liefting et al. 2008). Reductions in yield of 93% have been recorded in the United States (Munyaneza et al. 2008). Anderson (2008 cited in Teulon et al. 2009) reported that large yield losses of field grown potatoes have occurred in New Zealand, although it is unclear whether psyllid yellows or Lso was responsible. Recently, the same Lso pathogen has been recorded in carrots in Finland and the Canary Islands, associated with the psyllid species *T. apicalis* and *B. trigonica* (Munyaneza 2010, Alfaro-Fernández et al. 2012). Alarmingly, this is the first record of the Lso pathogen outside of the Americas and New Zealand, and not in a solanaceous plant (Munyaneza 2010).

Lso appears to have similar distributions in crops to TPP, being aggregated and occurring more at potato crop edges (Butler and Trumble 2012b, Martini et al. 2012). Lso has been documented in Texas to be clustered within the crop, with solitary infected plants between clusters (Henne et al. 2012). Findings from Workneh et al. (2012), found that Lso infections were greater at potato crop edges than within crop.

Lso development appears to be temperature sensitive (Munyaneza 2010). The temperature spectrum for Lso bacteria varies, with optimal development occurring at 28°C, while development is significantly slowed at or below 16°C, and above 35°C. Munyaneza (2010) has also observed that Lso development inside TPP has similar temperature dependencies.

1.4 Managing TPP and its associated host-plant plant symptoms

1.4.1 Monitoring for TPP

Effective TPP monitoring methods, such as sticky traps, have been developed for integrated pest management (IPM) programs in tomato glasshouse crops in the USA (Al-Jabr 1999). Sticky traps of different colours, orientations, and heights have been trialled to find the most effective combination. Fluorescent colours were the most effective, namely standard yellow, neon-green, and neon-orange. Traps were most effective when placed at the top of the plants, and should be orientated to receive direct sunlight. Although yellow sticky traps are an effective tool to detect adult TPP in potato crops, especially when they occur at low densities (Goolsby et al. 2007, Yen et al. 2012), economic threshold levels using this method are not yet reliably developed in the USA (Gharalari et al. 2009).

Sweep nets have been widely used for monitoring TPP in potato crops (Cranshaw 1994, Butler and Trumble 2012a). An index, based on the number of adults caught per 100 sweeps, has previously been correlated with the amount of psyllid yellows observed (Pletsch 1947,

Cranshaw 1994). Suction traps were trialled and found to be ineffective, only capturing a few TPP even when observed densities were high (Cranshaw 1994). Yellow pan traps have proven useful in detecting TPP migration movements (Cranshaw 1994), and have been recommended as a detection method for TPP in Australia (Yen et al. 2012).

More reliable sampling methodologies for TPP in potato crops have been recently developed. Martini et al. (2012) describes one method of removing TPP nymphs by washing the sampled potato leaves. Washing is done by rinsing the samples in cold water to remove sediment, submerging the leaves in hot (>85^{o}C) water for five seconds, then collecting the TPP nymphs by filtering the water using a fine mesh, and vacuum pump. Martini et al. (2012) then removed the mesh examining it under a stereo microscope to count the number of TPP. This method of sampling appears to be effective, both in time and effort required, and validates results found elsewhere on TPP spatio-temporal distributions (see Butler & Trumble 2012b).

Butler and Trumble (2012b) found that TPP has its highest densities at the crop edge, and, like Martini et al. (2012), found and aggregated distribution within the crop. This means there are patches of high TPP densities through the crop. They also attempted to develop an action threshold for when control action should be taken with the purpose of minimising economic losses caused by TPP. An action threshold is a critical tool in maximising the efficiency of insecticidal applications and reducing the spread of zebra chip by TPP (Gharalari et al. 2009, Butler and Trumble 2012b).

Yellow sticky traps have been utilised in monitoring potato crops in New Zealand for TPP (Cameron et al. 2009, Walker et al. 2011). The sticky trap method is heavily criticised by Martini et al. (2012) because the number of adult psyllids (the predominant life stage caught by traps) poorly correlates with nymph infestation levels in the crop, making the number of traps needed to be deployed potentially very expensive.

1.4.2 Chemical control of TPP

The establishment, spread, and impact of TPP and its vectored diseases has led to a disruption of established integrated pest management (IPM) protocols for all solanaceous crops in New Zealand (Teulon et al. 2009). Chemical control is presently the only proven method capable of providing efficient management of the zebra chip, by effectively killing TPP (Goolsby et al. 2007, Munyaneza 2010, Peng et al. 2011). However, it is recognised that long term, chemical control alone is unsuitable - due to factors such as the development of insecticidal resistance, disruption of current IPM programs, potential environmental contamination by

chemical agents, and market resistance. New IPM strategies must thus be developed (Gharalari et al. 2009, Flores-Dávila et al. 2011). In the interim, disruption of IPM has resulted in chemicals being researched and utilised to control TPP, consequently increasing insecticide use, with reduced reliance on natural enemies.

In the USA, a variety of insecticides have historically been used. Lime sulphur was the most successful early insecticide used in the 1930's and 1940's (Cranshaw 1994). Later, DDT, organophosphates, carbamates, and pyrethroids were used (Pletsch 1947, Cranshaw 1994).

A range of insecticides with different active chemical compounds have been tested in New Zealand and abroad (Berry et al. 2009, Gharalari et al. 2009, Nansen et al. 2011, Page-Weir et al. 2011). In laboratory bioassays in New Zealand, dichlorvos, lambda-cyhalothrin, methomyl, taufluvalinate, methamidophos and abamectin each had 98-100% nymph mortality rates 48 hours after application. Azadirachtin, spiromesifen, abamectin, spirotetramat and thiacloprid had 82-100% mortality 168 hours after application, while buprofezin, pymetrozine and imidacloprid (a drench) resulted in 36-53% nymph mortality rates after 168 hours (Berry et al. 2009). The results from Gharalari et al. (2009), who tested five compounds similar to Berry et al. (2009) (lambda-cyhalothrin, abamectin, spiromesifen, abamectin and pymetrozine) generally found higher mortality rates in a shorter time span, but this can be explained by the much higher concentrations of active compound used.

Insecticide control of TPP on capsicums in New Zealand has been further trialled by Page-Weir et al. (2011). Eleven insecticides, with a range of active compounds and properties were trialled with a variety of results. The compounds: abamectin + oil, bifenthrin, spiromesifen and spirotetramat gave the most effective control of TPP nymphs over the 6 week monitoring period. The application of Buprofezin + oil gave short term control for two weeks while the other insecticides gave inadequate control. Some insecticides, despite not causing high mortality rates, can have a repellent effect on TPP or reduce ovipositional rates and may still have a role in more holistic IPM programmes (Page-Weir et al. 2011).

Flores-Dávila et al. (2011) trialled a range of natural plant extracts, including one available in New Zealand (neem oil) as insecticidal treatments to control TPP. After 72 hours, neem oil caused mortality of 91-100% of nymphs at concentrations of 2,000 and 2,500 ppm. The most effective extract was from the seeds of *Annona muricata* ,which resulted in 95-100% nymphal mortality after 72 hours. Other plants extracts: *Thuja occidentalis*, *Sapindus saponaria*, *Euphorbia dentate* and *Carica papaya* were either relatively ineffective (<70% mortality), or required exceptionally high concentrations to reach acceptable mortality rates.

The effect of different insecticides on the behaviour of adult TPP, including the transmission of the bacterium responsible for zebra chip, have been investigated by Butler et al. (2011) in the USA. They tested three insecticides available in New Zealand: imidacloprid, abamectin and pymetrozine (see Berry et al. 2009, Page-Weir et al. 2011). Kaolin particle film and horticultural spray oil were also tested. All insecticides trialled reduced the duration TPP spent probing the leaf surface to feed and reduced the time spent on the leaflets. Imidacloprid (a systemic drench) and abamectin also significantly reduced the transmission of zebra chip disease. Imidacloprid has also been found by Butler et al. (2012) to reduce feeding duration and feeding events by TPP. Abamectin has been found to be an effective control measure for TPP previously (see Berry et al. 2009, Page-Weir et al. 2011), however, contrary to Butler et al. (2012), imidacloprid was found quite ineffective by Berry et al. (2009). The results indicate imidacloprid could still prove useful in reducing zebra chip occurrence but may not cause sufficient TPP mortality.

Bio-rational insecticides which act as repellents have also been trialled. Of the four insecticides trialled (SunSpray oil, BugOil, Requiem, or MOI-201), only SunSpray and BugOil are available in New Zealand. In both no-choice and choice tests, all four insecticides significantly repelled adults and deterred oviposition compared to controls (Yang et al. 2010b). The authors concluded there was a rank order in which the insecticides could be placed, from most effective to least: SunSpray oil > BugOil > Requiem > MOI-201.

A kaolin (platy aluminosilicate mineral) particle film was trialled on tomatoes in laboratory and field conditions as a non-toxic alternative to insecticide applications. Peng et al. (2011) found that when applied to both the upper and lower leaf surfaces, the kaolin film significantly reduced the number of eggs, and total psyllids per plant. Although there was a significant reduction, the number of TPP remained at 25-50 per plant, so other control methods would be required to integrate this potential management option (Peng et al. 2011). There are also concerns that kaolin film may interfere with natural enemies, disrupting IPM of other pests (Peng et al. 2011).

In the USA, an attempt has been made to optimise insecticidal applications to control TPP by developing a decision support (whether to spray or not) scheme. The decision is based on the likely success of the scheduled insecticide application (Nansen et al. 2011). Nansen et al. (2011) trialled four insecticides: abamectin, pymetrozine, spiromesifen and spirotetramat. These were trialled to see which application method (ground rig or fixed wing aircraft) provided the best coverage, how environmental variables affected spray coverage on the

plants, and how different levels of spray deposition affected TPP mortality rates. The authors concluded that such information is critical in minimising chemical costs to the grower, maximising spray effectiveness, and reducing associated negative environmental effects (Nansen et al. 2011).

1.4.3 Cultural control

There is very limited information on cultural control of TPP (Butler and Trumble 2012a). Some studies mention that earlier potato plantings can be more seriously affected by TPP than later planting in Montana, USA (Hartman 1937 in Al-Jabr 1999). The results imply later plantings are less affected, but this does not appear to be implemented as standard practice and refers to psyllid yellows rather than the new Lso threat. Coloured mulches on tomatoes have also been tested (Demirel and Cranshaw 2006). It was found that both aluminium mulch and white plastic mulches could significantly reduce the number of TPP found on the plants (Demirel and Cranshaw 2006). There has been some limited work on host resistance, but mainly in the context of tomatoes (Liu and Trumble 2004, 2005, Casteel et al. 2006).

Cranshaw (1994) suggested the use of bell peppers as a trap crop to attract the adult TPP to reduce adult colonisation on potato crops. Some weeds, such as nightshade (*Solanum* spp.), can act as an alternative host for TPP (see Martin 2008). Despite not being TPP's preferred hosts, it is conceivable that these weeds aid in the annual dispersal of TPP into crops. Therefore, controlling these weeds is likely to be a useful management tool (Cranshaw 1994). Many of these weeds are annuals, and thus do not provide an overwintering reservoir (Martin 2008).

1.4.4 Biological control

The three methods of biological control are: classical, augmentative and conservation - each represent differing approaches to managing a pest species. Classical biological control involves the importation of a pest organism's natural enemy(ies), from the pest organism's native range (Caltagirone 1981). The classical method has a long history of use and some well documented successes (Howarth 1991). Often, the pest organism has escaped its natural enemies when it was introduced to the non-native area, removing the regulating effect of natural enemies (Caltagirone 1981). By introducing these natural enemies, it is predicted that they will bring about a decrease in the pest population (Caltagirone 1981). The method can fail, however, to produce the desired results. Either the natural enemy fails to have an economic impact on the pest (Caltagirone 1981), or has non-target impacts, particularly on species which are native or endemic in either modified or natural ecosystems (Howarth 1991).

Augmentative biological control can be further subdivided into inoculative and inundative categories. Inoculative biological control entails rearing a natural enemy, releasing a small number to allow the population to increase over time (Waage et al. 1988, Corrêa-Ferreira and Moscardi 1996). Inundative biological control is where a large number of a natural enemy are reared and released, overwhelming the pest organism population and providing a relatively quick, large reduction in the pest organism's population (Waage et al. 1988, Collier and Van Steenwyk 2004). Augmentative biological control is generally an uncommon approach, only applied in a limited number of agricultural settings (Collier and Van Steenwyk 2004). Collier and Van Steenwyk (2004), found just 15% of case studies examined reduced pests below the required economic threshold, with this method of control often being less effective than agrichemical applications.

Conservation biological control (CBC) is where existing populations of natural enemies (either native or introduced) are enhanced through habitat engineering in an effort to increase their efficacy (Landis et al. 2000). Habitat manipulation includes providing access to food resources, alternative prey and/or hosts, and shelter from unfavourable conditions (Landis et al. 2000). The use of this 'environmentally friendly' pest control potentially reduces the need for agrichemicals. CBC has been shown by a number of studies to have potential benefits in helping with pest control (Jonsson et al. 2008). Conservation biological control has additional benefits, other than its potential pest control. The CBC initiative can be undertaken by a single grower, not requiring large-scale coordination and organisation like classical biological control (Jonsson et al. 2008). However, some habitat engineering, such as the addition of floral resources, can benefit the pests themselves or the hyperparasitoids of the beneficial insects (Baggen and Gurr 1998, Araj et al. 2009, Araj et al. 2011). Studies demonstrating conservation biological controls benefits to the actual producers/growers in terms of savings or increases in profit are limited (Jonsson et al. 2008).

1.4.5 Biological control and TPP

Classical biological control in the context of potatoes is very limited to date. In New Zealand, a parasitoid (*Tamarixia triozae* Burks) of TPP is being trialled in quarantine for non-target effects on native psyllids, and its effectiveness at parasitising TPP evaluated for potential release (Workman and Whiteman 2009). *Tamarixia triozae* is generally considered an inefficient biological control agent, with low parasitism levels, poor synchronisation with TPP populations, and ineffective dispersal (Pletsch 1947, Butler and Trumble 2012 and references within). A native parasitoid, an undescribed species of *Tamarixia*, is also being tested to evaluate its effectiveness as a biological control agent (Workman and Whiteman 2009).

An augmentative biological control approach using entomopathogens was trialled by Lacey et al. (2009). The entomopathogens are commercially available and were tested to determine their potential to control TPP nymphs and adults on potatoes under ideal conditions (25^{o}C) for the fungi. The researchers used five isolates of three different entomopathogens (*Beauveria bassiana*, *Metarhizium anisopliae* and *Isaria fumosorosea*). The two isolates of *M. anisopliae* and *I. fumosorosea* both provided 95-99% mortality rates of TPP adults after 2-3 days and 91-99% mortality in nymphs in 4 days post-application. *Beauveria bassiana* caused a relatively low mortality rate of 53% on adults and 78% on nymphs, both after 4 days.

Lacey et al. (2011) further evaluated two of the previously studied entomopathogens, *M. anisopliae* and *I. fumosorosea*. *Metarhizium anisopliae* was trialled at three concentrations, with a maximum mortality rate of 67% compared to 63% with the insecticide abamectin. *Isaria fumosorosea* was tested at one concentration and also in combination with neem oil, achieving 78% and 76% mortality respectively, while abamectin achieved 84%. Only *I. fumosorosea* and abamectin achieved significant reductions in plant damage which included zebra chip symptoms. *Isaria fumosorosea* combined with neem oil and abamectin significantly increased tuber yields. The two entomopathogens were then trialled together, both at two different concentrations. *Metarhizium anisopliae* achieved a maximum mortality rate of 62% and *I. fumosorosea* 66%. The highest concentration of *M. anisopliae* achieved a significantly higher tuber yield than the control, while both concentrations of *I. fumosorosea* had significantly higher yields than the control. Both entomopathogens significantly reduced plant damage and zebra chip symptoms and therefore, therefore having potential for implementation by growers as a control method.

Al-Jabr (1999) evaluated two lacewing species (*Chrysoperla carnea* Stephens and *Chrysoperla rufilabris* Burmeister) in Colorado, USA for TPP management. Although commonly occurring predators, it was unknown if these particular species fed on TPP. Experiments proved these lacewings do consume TPP, and that they could complete their lifecycle on a diet exclusively of TPP. *Chrysoperla carnea* consumed more TPP nymphs than *C. rufilabris*, but had a longer development time. A field evaluation of *C. rufilabris*, by releasing its eggs into a TPP infested potato crop did not lead to a reduction in TPP nymph densities.

A number of natural enemies that have been the target of conservation biological control studies in other agricultural and horticultural settings (e.g. Irvin et al. 1999, Jonsson et al. 2009) have been identified as common and important predators of TPP in potato crops in New

Zealand. Predators of TPP that occur naturally in potato crops in New Zealand are brown lacewing (*Micromus tasmaniae* Walker), small hoverfly (*Melanostoma fasciatum* Macquart), 2-spot ladybird (*Adalia bipunctata* L.) and the 11-spot ladybird (*Coccinella undecimpunctata* L.) (Jorgensen et al. 2011, Walker et al. 2011). These predators have relatively low consumption rates in comparison to the southern ladybird (*Cleobora mellyi* Mulsant), though they can be present in high densities (Walker et al. 2011). This difference in consumption rates could be due to differences in predator size. To date the naturally present natural enemies have failed to control TPP numbers in mid and late summer when it is ideal for TPP development (Walker et al. 2011). Integrated pest management (IPM) is currently in the early developmental stage in regards to incorporating TPP (Goolsby et al. 2007, Gharalari et al. 2009).

1.5 Ladybirds

Ladybirds (Coccinellidae) contain a large number of formally described species, and a worldwide distribution (Hodek 1967). They are holometabolous insects, having four distinct life stages: egg, larva, pupa, and adult (Hagen 1962). They are generally considered to be beneficial insects, feeding on pest species such as psyllids, aphids, scale insects, whitefly, mealybugs, mites, and small Lepidoptera and Coleoptera larvae (Hagen 1962, Hodek 1967, Obrycki and Kring 1998). They are also recorded feeding on nectar and pollen (Hagen 1962, Hodek 1967). An exception is the herbivorous Epilachninae subfamily, which can be pests in agricultural and horticultural settings (Hagen 1962). In some instances, ladybirds introduced for biological control purposes have become an invasive species, displacing native ladybirds (Ware and Majerus 2008). Their introduction can disrupt existing biological control through intraguild predation or consumption of aphid mummies (Rosenheim et al. 1995, Obrycki and Kring 1998).

Coccinellids have been utilised as biological control agents for over a century, with ladybirds being utilised in all three methods of biological control (Obrycki and Kring 1998). There have been some spectacular successes using ladybirds as biological control agents, but methods to enhance their efficacy, in relation to CBC, are under developed (Obrycki and Kring 1998). Indeed, coccinellids could be considered crucial in developing an integrated approach to pest control (Obrycki and Kring 1998).

Non-prey foods are an important and often integral part of predacious ladybirds' diets (Lundgren 2009). Mixed diets, using prey and non-prey foods, often strongly enhance larval performance, and adding sugar to adult diets considerably enhance adult performance and

reproduction compared to prey only diets (Lundgren 2009). However, a previous study by Spellman et al. (2006), using buckwheat (*Fagopyrum esculentum* Polygonaceae) as a floral resource (nectar and pollen) for the ladybird *Harmonia axyridis* Pallas found it did not lead to a reduction in the number of the spirea aphid (*Aphis spiraecola* Patch) under glasshouse conditions.

1.5.1 Southern ladybird

The southern ladybird (*Cleobora mellyi* Mulsant) (Coleoptera: Coccinellidae) was originally introduced to New Zealand in the 1970s, as a biological control agent for the pest eucalypt tortoise beetle (*Paropsis charybdis* Stål.), a major defoliator of plantation *Eucalyptus* spp. (Baker et al. 2003, Berndt et al. 2010). The ladybird is endemic to Australia, where it is distributed throughout the southern and central parts of the continent (Slipinski 2007). The southern ladybird feeds predominantly on the larvae of chrysomelids and hemipterous insects such as aphids and psyllids (Baker et al. 2003, Murray et al. 2008, Slipinski, 2007). Augmentative releases of the southern ladybird for biological control of chrysomelids are considered a possibility in Tasmania. (Bashford 1999, Baker et al. 2003).



Figure 3. Adult southern ladybird on a potato leaflet.

The southern ladybird has also been investigated as a biological control agent of the chrysomelid, *Chrysophtharta bimaculata* Olivier. This chrysomelid species is a major defoliator of *Eucalyptus* spp. in Tasmania (Elliott and Little 1980). Mensah and Madden (1994) found that the southern ladybird has good potential to help control *C. bimaculata*, with the main limiting factor being alternative food when *C. bimaculata* larvae reached 3rd to 4th instar and became too large for the southern ladybird to consume. However, access to supplementary food in the form of sucrose spray or granules increased the number of southern ladybirds present and increased their efficiency. The study indicates the potential importance

of floral resources with regards to the southern ladybird and biological control of TPP. Since the southern ladybird must be released into crops in New Zealand, extra floral resources may assist in keeping the southern ladybird at its release site in a potato crop. It has also been observed that the southern ladybird will utilise buckwheat as a food source (O'Connell, Unpublished data).

There have been further releases of the southern ladybird since its original introduction. A release of 3000 adults and 1350 eggs was made at sites in the northern and central North Island in an attempt to increase its range (Murray et al. 2008), with previous surveys finding that the southern ladybird had not dispersed far from its original release site in the Marlborough Sounds (Murray et al. 2008). Further releases occurred in 2005 and 2007, with surveys of these areas finding the southern ladybird well established around New Zealand (Berndt et al. 2010).

The southern ladybird has a close association with several Australian psyllid species (Mensah and Madden 1994, Withers 2001). Many of these psyllid species found in New Zealand, have been accidentally or self-introduced, inhabiting *Eucalyptus* spp. trees (Withers 2001). Psyllids are considered essential to the diet of the southern ladybird in order for it to complete its lifecycle (Bain et al. 1984).

Coccinellids usually enter diapause or aestivation when environmental conditions or food availability and quality become poor, changes generally associated with seasonal variation (Hagen 1962, Hodek 1967). Observations of the southern ladybird show when provided with sufficient food, there is no noticeable seasonal change in its development rates or a reduction in reproduction through seasonal changes when being cultured under natural light (John Thompson, pers. comm. BioForce Ltd. 2012). This has important implications for possibly sustaining southern ladybird populations between growing seasons and allowing the southern ladybird population to closely synchronise with seasonal pest population fluctuations.

Only one natural enemy of the southern ladybird is known to occur in New Zealand, the parasitoid *Dinocampus coccinellae* Schrank. It has been recorded as parasitising the southern ladybird in Tasmania, Australia (Ceryngier et al. 2012). This parasitoid has a cosmopolitan distribution and has been recorded as parasitising a number of ladybird species (Ceryngier et al. 2012). It is not clear what occurrence rate *D. coccinellae* has in New Zealand, nor what regulatory effect (if any) it may have on the southern ladybird.

1.6 Aims and objectives

This project is based upon the new species biological control concept, wherein the natural enemy and the pest have no evolutionary history; thus the natural enemy should be more effective at controlling the pest than instances where the natural enemy and pest have co-evolved. (Hokkanen and Pimentel 1984, 1989). The new species association is based on taking advantage of the lack of evolved commensalism found in many predator-prey systems (Hokkanen and Pimentel 1989, Pimentel 1991). New species associations have proven to be effective at controlling pest species in many instances (Hokkanen and Pimentel 1984, 1989) and have a success rate approximately three times higher than old-association control (Pimentel 1991). Laing and Hamai (1976 cited in Hokkanen and Pimentel 1984), found a 75% greater success rate if a parasite (including predators) and its host were newly associated, compared to old-association biological control programmes.

In order to assess the potential of the southern ladybird as a biological control agent of TPP, it is necessary to investigate key aspects of its ecology. Very little is currently known about the southern ladybird and TPP predator-prey dynamics; only the ladybirds' voracity and behavioural responses with TPP have been studied (O'Connell et al. 2012). No knowledge currently exists on its ability to reduce TPP densities, prey selection (in this context), or longevity in a New Zealand Solanaceae crop. Therefore, to give important insight to the southern ladybird's potential as a biological control agent of TPP, and determine if future augmentative releases into potato crops could be useful, the following objectives were addressed experimentally:

1) Assess if the southern ladybird can reduce TPP densities on potato plants.

2) Examine if the southern ladybird has a prey preference among common herbivorous potato pests.

3) Investigate the longevity of the southern ladybird when presented with a floral resource and/or low TPP provision.

Chapter 2

Does *Cleobora mellyi* reduce TPP densities in a glasshouse microcosm?

2.1 Abstract

Currently there is a reliance on insecticides to manage the tomato-potato psyllid in New Zealand. The overuse of insecticides may eventually lead to resistance in the TPP, making spraying less effective over time, in addition to any environmental harm, consumer 'resistance', and chemical costs to growers. Therefore, it is important to develop alternative control strategies such as biological control to provide effective management of TPP. This chapter examines if the southern ladybird can reduce TPP densities in a glasshouse grown potato crop, and if this leads to a trophic cascade. During 10 weeks of sampling, the southern ladybird significantly reduced TPP densities on potato plants, causing an 84% reduction. Plants with only TPP and no predator failed to produce any tubers, while those with the ladybird did produce tubers. However, the quantity of tubers was significantly reduced (by 60%) where the ladybird was present with a reduced size compared to both the control and ladybird only plants. There was no difference in dry matter content between treatments that produced tubers.

2.2 Introduction

Historically, in the USA, chemicals have been the method of choice by growers for controlling TPP, with the compounds used changing over time (Cranshaw 1994). Since the arrival of TPP in New Zealand, a range of insecticides have been trialled to determine their effectiveness against it (e.g., Berry et al. 2009, Page-Weir et al. 2011), but the use of insecticides, even if properly managed can still lead to the build-up of resistance to the active compound. Therefore, such control strategies must be augmented or replaced with other management methods, such as biological control.

Effective biological control requires a natural enemy to kill enough of a pest to keep its density below a known threshold, at a particular time (Rochat 1997, Hoelmer and Kirk 2005). The threshold is usually calculated based on economic criteria (Stern 1973, Rochat 1997). To understand and test if a predator could reduce a given pest below an economic threshold, it is important to have an understanding of the predator-prey dynamics (Dixon 2000).

Concerted efforts have been made to model these interactions in a range of study systems (Dixon 2000, Evans 2008, Kean et al. 2008), systems involving ladybirds have failed to thoroughly investigate the role of alternative concepts such as optimal foraging theory (Dixon 2000). Many of these theoretical studies provide lists of desirable traits for biological control agents (Dixon 2000), but these lists are often too general to be of use for biological control practitioners (Dixon 2000).

Little is known about ladybird-psyllid population dynamics because psyllids are often considered less important prey for ladybirds than other herbivores such as aphids (Hodek and Honěk 2009). It has been observed that *Olla v-nigrum* (Mulsant) ladybird populations increased in response to Asian citrus psyllid (*Diaphorina citri*) presence (Michaud 2001). But this study failed to determine if this interaction had any effect on the psyllid density. Often studies investigating ladybirds as biological control agents of psyllids only investigate consumption rates, behaviour, prey choice, life-cycles and development rates (e.g. Mehrnejad and Jalali 2004, Pluke et al. 2005, O'Connell et al. 2012), failing to answer important questions about predator-prey dynamics, particularly of ladybird larvae (Dixon 2000).

The generation time ratio between predator and prey, ladybird and psyllid can have a large influence on predator-prey dynamics by allowing the predator population to more closely match the prey population fluctuations (Kindlmann and Dixon 1999; 2001). Where ladybirds have a generation time or population growth rate similar to or greater than that of the prey, the ladybirds are likely to be able to regulate the prey population (Dixon et al. 1997, Dixon 2000). Even where a prey species has a faster population growth rate than that of its predator, the latter could still regulate the prey if its voracity is high enough (Dixon et al. 1997).

However, although often a predator's slower developmental time, relative to its prey may mean that the predator fails to have a substantial impact on prey abundance (Kindlmann and Dixon 1999; 2001). Potential augmentative releases, particularly if these are inundative, could overcome this barrier to effective control (Parella et al. 1992, Obrycki et al. 1997).

The predator-prey interaction between the southern ladybird and TPP is novel and has only been previously investigated to determine maximum daily consumption and ladybird behaviour in the presence of TPP (O'Connell et al. 2012). While this is important information, further testing prior to a field release is critical. It is important to test new biological control agents for their efficacy in semi-field, or field cage conditions (Hoelmer and Kirk 2005). This enables a degree of certainty about whether the biological control agent will be effective at limiting the pest (Hoelmer and Kirk 2005) which could lead to a trophic

cascade. If the biological control agent is not effective at reducing pest densities, it should not be released as a precaution to prevent unintended consequences (Hoelmer and Kirk 2005).

Trophic cascades have been widely demonstrated in aquatic and terrestrial ecosystems (Strong 1992, Halaj and Wise 2001, Shurin et al. 2006). They occur by indirect positive effects from predators on plant biomass mediated by herbivore suppression (Halaj and Wise 2001, Mooney et al. 2010). It is a concept that has long been recognised (Halaj and Wise 2001), with reports of top-down cascading effects dating back 2,500 years (Begon et al. 1996). Halaj and Wise (2001) undertook a meta-analysis of generalist predacious arthropods (such as ladybirds) found that the effects of a trophic cascade tend to be stronger in crop systems than in more natural systems. Halaj and Wise (2001) conclude that omnivory or intraguild predation, which is common among ladybirds, does not prohibit the predator from a trophic cascade occurring by reducing predator effectiveness.

The objective of this chapter was to: 1) in a glasshouse microcosm investigate the potential of the southern ladybird to reduce TPP densities on potato plants; 2) investigate if a trophic cascade occurred resulting in increased tuber quality; 3) provide some insight to the predator-prey dynamics of this model system. The hypothesis was that the southern ladybird would reduce TPP densities but that this would not lead to a trophic cascade due to the Lso bacterium.

2.3 Methods

2.3.1 TPP and southern ladybird

The TPP used in this trial were cultured on potato plants (cv. Moonlight) and tested for Lso prior to being used. All TPP cultures tested positive, and the culture with the lowest titre value was selected as the inoculant culture. The southern ladybird individuals were obtained from Bioforce Ltd (Auckland, New Zealand), having been reared on a mixture of TPP and green peach aphid.

2.3.2 Experimental design

This trial was a 2x2 factorial in a randomised complete block design with a control and three treatments: 1. Potato plant only, 2. Potato plant + southern ladybird, 3. Potato plant + TPP, 4. Potato plant + TPP + southern ladybird. There were six replicates of each treatment. Insect dome cages (Bugdorm insect rearing tent, Taiwan) were used to ensure independence between experimental units. Each cage had a potted potato plant in a 5 L plastic container with a saucer. The potatoes were planted in a potting soil that came from a 500 L batch consisting of

400 L bark, 100 L pumice, 1500 g Osmocote Exact Standard (3-4 month), 500 g horticultural lime, and 500 g Hydraflo. Treatments that contained TPP were inoculated with 50 adult TPP when the plants were approximately 15 cm tall (13 days after planting). Data loggers (iButton, Maxim Integrated Products Inc., USA) were used to record temperature once every hour and determine when approximately one generation of TPP had been completed based on development times from Tran et al. (2012). In this case, it was approximately 27 days for one generation to be completed based on Tran et al. (2012) study . The appropriate treatments then had two southern ladybird adults released into each cage – at this stage the plants are 40 days old. The data loggers were then paired, one inside the cage, one outside and these pairs randomly assigned to one cage in each block to test for any temperature differences the cages may have created.



Figure 4. The layout of the dome cages for the glasshouse experiment. Insert: an iButton attached to the outside of a dome cages, and the back of the iButton inside the cage.

2.3.3 Sampling and harvesting

Weekly assessments of TPP densities were made by sampling a middle leaf from a randomly selected stem, which is a recently developed reliable sampling method (see Butler & Trumble 2012b, Walker et al. 2012). The number of nymphs and adults was counted and recorded. The position on the plant of the ladybirds was also recorded (off plant, lower, middle, upper) to determine if they had a 'preferred' foraging zone. Concurrently, any ladybird eggs were removed to ensure there was no additional predation from hatched ladybird larvae. This was done for 10 weeks before sampling had to be terminated due to symptoms of psyllid yellows

and zebra chip disease causing plant die-off in treatment's three and four. The rapid die-off between weeks nine and 10 left very little green foliage, and plant senescence was complete seven days later. At week 10, all ladybirds were removed from the treatments – one hundred and three days after planting.

One hundred and forty days after planting, the plants and their tubers were harvested, as this is when the plants haulms are expected to reach maturity (Anderson et al. 2004). The tubers were washed and dried, and then the number of tubers in each pot was counted. The tubers in each pot were weighed together, and 10 days later, their specific gravity measured (Fig. 4). Specific gravity is used to calculate percentage dry matter in tubers. For the 10 days between harvest and specific gravity measurement, the tubers were stored at 8 ^{o}C.

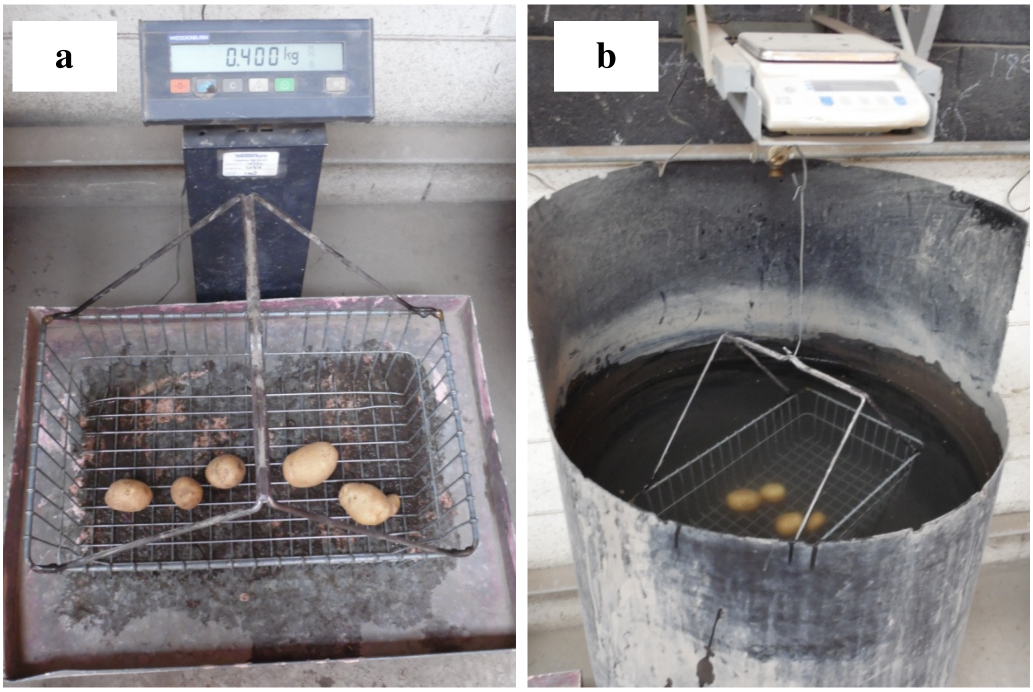


Figure 5. Weighing the potato tubers first as normal (a) and then weighed suspended in water (b), to provide the data to calculate specific gravity, which is used to determine percentage dry matter.

2.3.4 Data analysis

The density of TPP, tuber number, fresh weight, and percentage dry matter was analysed using a general ANOVA in Genstat 14 (VSN International). The TPP density data was transformed using square root before analysis, and fresh weight transformed by $\log_{10}$. Significance between treatments was determined using 5% LSD. After analysis the means

were back-transformed. Treatment three had to be excluded from final analysis of potato tuber metrics because no tubers were produced in this treatment.

2.4 Results

There were no significant differences in TPP density at the time of ladybird release. By the final week, there was a highly significant difference (df=3 P=<0.001) in TPP densities between treatments. Where there was ladybirds and TPP, there was significantly fewer TPP than only TPP (Table 1). Using the back-transformed means to calculate TPP densities, after 10 weeks, treatment four showed a significantly lower density of only 15.7% the number of TPP compared to treatment three. There was no significant effect of blocking.

Table 1. Comparisons between the treatments that had TPP at the ladybird release, and then again at the final TPP sampling. Data is presented as square root transformed, and back-transformed means. Treatment three was TPP only, treatment four was TPP + ladybird.

Treatment	TPP week 1	Back-transformed means	TPP week 10	Back-transformed means
T3	7.23	52.27	7.70	59.29
T4	7.53	56.70	3.05	9.30
LSD value	4.75	-	1.66	-
Significance	ns	-	P=<0.001	-

The number of tubers differed significantly between treatments (df=3, P=<0.001). In the presence of ladybirds and TPP (T4) the plants had significantly fewer tubers compared to the control and plants with ladybirds only (Table 2.). The mean fresh weight of these tubers was also significantly different (df=3, P=<0.001 with treatment four tubers weights significantly less compared to treatments one and two. There was no difference between treatments for dry matter.

Table 2. The mean number of tubers, mean fresh weight, and mean percentage dry matter for each treatment with back-transformed means. Fresh weight is transformed log_{10}. Treatment one was the control, treatment two the ladybird only, treatment three TPP only, treatment four TPP + ladybird.

Treatment	Mean number tubers	Significance	Log_{10} mean fresh weight (kg)	Back-transformed mean fresh weight (kg)	Significance	Mean % Dry matter	Significance
T 1	5.00	ns	-0.55	0.37	ns	13.98	ns
T 2	4.67	ns	-0.574	0.36	ns	15.06	ns
T 3	0	-	-	-	-	0	-
T 4	2.00	P=<0.001	-1.586	0.06	P=<0.001	13.88	ns
LSD value	1.07		0.1380			3.129	

Of the four categories, the ladybirds were most often seen in the middle section, but there does not appear to be any clear preference for which part of the plant is the southern ladybird's most preferred foraging area (Fig 6). The ladybirds were observed on the plant a total of 75% of the time, and a total of 51% of the time in the middle and upper parts of the plant.

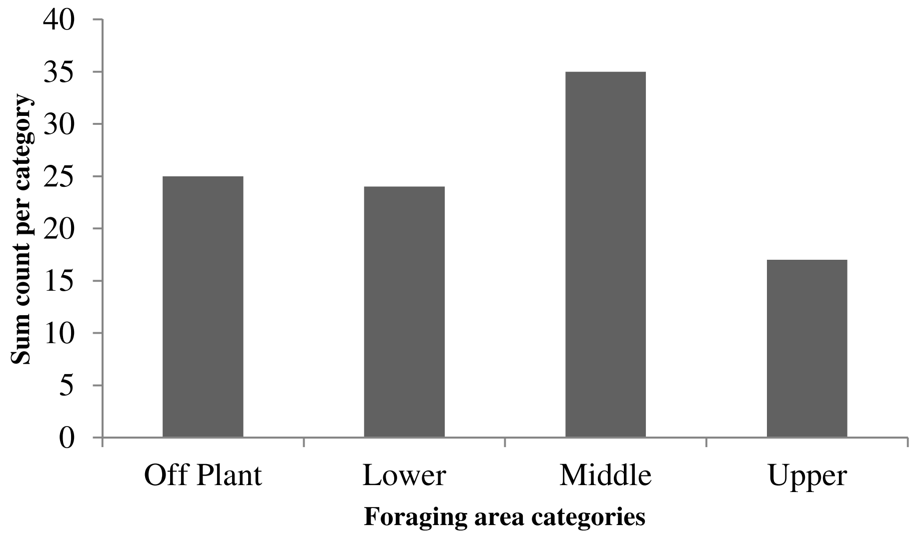


Figure 6. The number of times ladybirds were observed feeding in each of the four categories in treatment four (ladybird + TPP).

No differences in temperatures between inside and outside dome cages or a difference between blocks was found. The overall mean for temperature from TPP inoculation to harvest was 21.92 ±0.3 ^{o}C.

2.5 Discussion

This study showed that ladybird-psyllid interactions resulted in a significant reduction in psyllid densities, as was hypothesised. In fact, very little is known about ladybird – psyllid interactions as evidenced by only two paragraphs in the Hodek and Honěk (2009) review on ladybird prey. Finding that the southern ladybird reduced TPP densities on potato plants has potential importance. A study by Ogol and Spence (1997) found, despite a marked increase in the number of ladybirds present correlated to increases in the leucaena psyllid (*Heteropsylla cubana* Crawford), no effect was found on sampled psyllid numbers. The Malaysian metallic blue ladybeetle (*Curinus coeruleus* Mulsant) reduces the population size of the Asian citrus psyllid (*Diaphorina citri*), but only when the psyllids' host plants were not pruned or treated with insecticide, whereby the ladybird was ineffective (Soemargono 2007).

A species-level trophic cascade (see Polis et al. 2000) was found, in contrast to what was hypothesised. There were significant differences in tuber number and fresh weight where there was ladybird + TPP compared to ladybird only and control (Table 2). Where there was only TPP, no tubers were produced (Table 2). Finding tubers (although reduced in number and weight) with ladybird + TPP, and no tubers with only TPP is indicative of a species-level

trophic cascade. However, it is difficult to determine the cause of such a cascade occurring due to a confounding factor, the presence of Lso vectored from the TPP.

No tubers being produced in treatment three is possibly due to higher TPP densities in the absence of the predator (Table 1). However, all these plants also reached mortality after 10 weeks of TPP sampling (104 days of growing), exhibiting severe symptoms of psyllid yellows or zebra chip disease on the foliage. This premature die-off is consistent with other studies where potato plants have been infected with Lso (Munyaneza et al. 2008, Gao et al. 2009).

Premature die-off of the plant caused by Lso does not solely explain why plants with TPP only did not produce tubers, and plants with ladybirds and TPP did. Gao et al. (2009) found in potato plants infested with TPP that were positive for Lso resulted in a reduction in the plants photosynthetic rate, a common effect of arthropod infestations (Peterson and Higley 1993). In the present study, reduced photosynthetic rate could have influenced tuber development via reduced carbohydrate production. This alone does not explain the difference, because one would expect both treatments to fail to produce tubers. In the presence of TPP and ladybirds, after 10 weeks only 50% of the plants had senesced, indicating that perhaps the difference in TPP densities between treatments three and four did contribute to the trophic cascade.

The reduction in plant stress from less TPP pressure possibly allowed tuber development. Herbivory can alter and interfere with the allocation of resources to a photosynthetic sink (Trumble et al. 1993), so the reduced TPP pressure may have interfered less with the process of tuber formation. Feeding by the aphid *Aphis nasturtii* Kalt. on potato plants decreases the size, but not the number of tubers (Bardner 1966 cited in Bardner and Fletcher 1974). Plants can also compensate for herbivore damage (Trumble et al. 1993, Baldwin and Preston 1999), but if such a response occurred, it appears to have been overwhelmed by the combination of Lso and TPP in treatment three.

Despite the differences in tuber number and weight, the tubers did not differ in their percentage dry matter (Table 2) as calculated from their specific gravity. This is in contrast to the work of Liefting et al. (2008) who found a significant reduction in dry matter of potato tubers infected with Lso, although the reduction in yield was consistent with the results presented here. Other factors such as the sugar content of potato tubers also need to be considered depending on the intended use. For example tubers to be processed and fried require a low sugar content to reduce unwanted browning (Kumar et al. 2005). The small tuber size produced in treatment four meant they were unable to be fried to examine for symptoms of zebra chip disease.

Where ladybirds could feed on TPP, 83% of the ladybirds survived the 10 week trial. In contrast there was 100% ladybird mortality where they had no prey by the second week of the experiment. This is encouraging that the southern ladybird can readily survive on a diet consisting only of TPP, with a low predator mortality rate.

Different species of ladybird have different preferences for where they predominantly forage on a plant. An example is the ladybirds *Coleomegilla maculata* (DeGeer) and *H. axyridis* in sweetcorn (*Zea mays*). *Coleomegilla maculata* as an adult fed lower on the plants than *H. axyridis* (Musser and Shelton 2003). It may not be surprising that there was no clear preferred foraging area on potato plants by the southern ladybird, due to it having evolving in a different plant architecture type in Australian eucalyptus forests. The sampling of the foraging areas was not particularly intensive, and as such should only be considered a preliminary indication of foraging habit on potatoes.

2.5.1 Conclusions and future research

The significant reduction in TPP densities from the presence of the southern ladybird over 10 weeks indicates that this ladybird could be a useful biological control agent for TPP, even in settings where high background levels of TPP exist.

Although a large reduction in TPP numbers was found in the presence of the ladybird, there was a corresponding reduction in the number and size of tubers produced. This was an improvement on where there was no ladybird to control the TPP, but the much reduced tuber number and size means the success of reducing TPP densities may only have limited economic/applied applications. If a threshold of TPP densities is developed, this will help shed light on the suitability of the southern ladybird as a biological control agent for TPP

TPP is predominantly found in the middle to upper parts of potato plants (Butler and Trumble 2012a, Martini et al. 2012), where the southern ladybird was observed 51% of the time. So despite only being observed to be in the parts of the plant which most likely had the highest TPP densities just over half of all observations, the significant reduction in TPP density was recorded.

The proof of concept that TPP densities can be reduced by predation from the southern ladybird has been demonstrated. The next step in assessing this predator's ability to manage TPP is to perform either field cage trials, or small scale field releases of the ladybird. Other options could include work on solanaceous glasshouse crops such as capsicums or tomatoes.

Chapter 3

Prey preference of the southern ladybird on potatoes

3.1 Abstract

Prey preference is an important aspect when assessing generalist biological control agents. Using a laboratory-based experiment, this study assessed the prey preferences of adult and larval southern ladybird on potato plants. In small experimental arenas, the existence of prey preference was tested by presenting ladybirds with a choice between TPP nymphs and other small herbivore pest nymphs (green peach aphid, potato aphid, or whitefly). Neither adult nor larval ladybirds showed a preference when presented with either green peach aphid or potato aphid as a choice. Both adult and larval ladybirds showed an aversion to whitefly, consuming significantly fewer whitefly. None of the treatments showed a significant difference in the number of TPP consumed

3.2 Introduction

Coccinellidae are commonly used as biological control agents on small herbivorous pests where aphids are their predominant target prey (e.g., Wratten 1973, Dixon et al. 1997, Obrycki and Kring 1998). However, in some instances, ladybirds are also used to control pest Coleoptera or Lepidoptera (Obrycki and Kring 1998, Berndt et al. 2010). To that end, the southern ladybird was introduced to New Zealand as a biological control on the eucalyptus tortoise beetle (Berndt et al. 2010). The southern ladybird's polyphagous diet (Slipinski 2007) allows for further investigation as a potential biological control agent of other pest insects, such as on tomato-potato psyllid in potato crops.

Measuring a biological control agent's voracity is critical to assess its potential for regulating the prey population (Lucas et al. 1997). O'Connell et al. (2012) investigated the southern ladybird's voracity and feeding behaviour on TPP in small experimental areans, finding that ladybird adults and larvae could consume up to 100 TPP in 24 hours, and ladybird adults spent significantly more time consuming TPP on potato leaflets compared to tomato leaflets. A key consideration when measuring predator–prey responses of a generalist predator is assessing that predator's response in the presence of alternative prey. If more than one suitable prey type is present in the crop, this may reduce the inference of the consumption results (Lucas et al. 1997).

New Zealand has a variety of phloem-feeding herbivore pests in potato crops with potential to be alternative prey for the southern ladybird. Aphids in particular are major pests of potatoes in New Zealand and worldwide (Stufkens and Teulon 2001, van Toor et al. 2008). The green peach aphid (*Myzus persicae* Sulzer), is polyphagous, vectoring potato leaf roll virus (PLRV) and potentially other viruses (Stufkens et al. 2000, Stufkens and Teulon 2001). Two other aphid species, the potato aphid (*Macrosiphum euphorbiae* Thomas) and foxglove aphid (*Aulacorthum solani* Kaltenbach) commonly occur in potato crops across Canterbury, vectoring PLRV and other potato viruses (Stufkens et al. 2000). Stufkens & Teulon (2000), demonstrated that these three aphid species made up >96% of the total number of aphids sampled in Canterbury, with the green peach aphid accounting for 85% of all aphids found. In New Zealand, up to nine species of aphids are known to transmit PLRV, but only the green peach, potato and foxglove aphid are commonly found (Stufkens et al. 2000).

Another potential alternative prey species of the southern ladybird, the greenhouse whitefly (*Trialeurodes vaporariorum* Westwood), is an occasional pest in field potatoes, but is more commonly associated with solanaceous glasshouse crops (Martin 1985), such as tomatoes and capsicums. In glasshouse cucurbit crops, *T. vaporariorum* vectors beet pseudo-yellows virus disease (Clover et al. 2002). The greenhouse whitefly causes losses in both cucurbit and solanaceous crops by excreting a sugary residue which facilitates sooty mould growth on leaves (Martin 1985).

TPP and aphids substantially overlap in their spatiotemporal distributions in Canterbury field crops (pers. obs.) an overlap most likely occurring throughout New Zealand, based on current and historical data on TPP and aphid population dynamics (Stufkens and Teulon 2001, Potatoes NZ 2012). Clearly, should the southern ladybird be strategically released into a field crop, it will inevitably be presented with more than one potential prey species. Therefore, choice tests are important to assess whether or not the southern ladybird has a preference for TPP, which would be the target pest of this ladybird's release for biological control purposes.

Choice tests are most often used to assess the preferred prey of predacious insects (Browne and Withers 2002). These tests are standard practise when assessing new organisms for release in New Zealand for the purpose of biological control (Barratt and Moeed 2005). Subsequently, because the southern ladybird requires releases into potato crops, it is critical to know which herbivorous prey it prefers, if any.

The southern ladybird has not encountered either of the aphid species or the whitefly species until recently in its evolutionary history; because the southern ladybird is native to Australia

(Slipinski 2007) and the green peach aphid, potato aphid, or greenhouse whitefly are comparably recent arrivals. The southern ladybird is closely associated with other psyllid and aphid species in its native range of Australia (Mensah and Madden 1994, Slipinski 2007), and has previously been shown to have a prey preference for Acacia psyllids (*Acizzia* spp.) over eggs of the eucalyptus tortoise beetle in choice tests (Mansfield and Bain n.d.).

The aim of this study was to evaluate whether the southern ladybird preferred particular prey species when presented with a choice of TPP and other herbivorous insects on potato leaflets in small experimental arenas. Both ladybird adults and larvae were studied separately using the same experimental design. The alternative prey species provided were green peach aphid nymphs, whitefly nymphs, and potato aphid nymphs. I hypothesised that the southern ladybird would not show a prey preference for TPP over green peach aphid, potato aphid, or whitefly.

3.3 Methods

3.3.1 Insect culturing

All four prey species were reared on potato (*So. tuberosum* cv. desiree) for multiple generations. The whitefly was initially collected from a field cage at Lincoln University where potato plants were growing, and the potato aphid from a private potato crop in Lincoln, New Zealand. The green peach aphid and TPP came from established laboratory cultures. The ladybirds were obtained from Bioforce Ltd New Zealand, having been reared on a mixture of TPP and green peach aphid. Prior to the experiment, ladybirds were maintained on a mixture of pea aphids (*Acyrthosiphon pisum*), green peach aphid, potato aphid, whitefly and TPP to ensure the ladybirds had experience with all prey presented in the choice test. The ladybird larvae used were a mixture of 3rd and 4th instar, randomly assigned to an experimental arena. These particular larval instars were chosen for this experiment based on their availability, the ease with which they could be handled and to be consistent with O'Connell et al. (2012). Using these instars allows to the work of O'Connell et al. (2012). All insects were maintained at 24^{o}C and 16:8h light: dark before and during the experiment. The ladybirds were starved for 24 hours prior to the commencement of the experiment to standardise their level of hunger, with a moistened dental roll provided to prevent desiccation.

3.3.2 Experimental design

The experiment comprised three treatments: 1) TPP + green peach aphid, 2) TPP + whitefly, 3) TPP + potato aphid. Small plastic experimental arenas (8.5 cm x 2.5 cm) with moist filter

paper in the base were used in a controlled environment room (24°C ±2°C). These arenas were slightly modified by removing the plastic ribs under the lid, which have previously been observed to allow enough space for TPP to escape. Two similar-sized potato leaflets (grown in insect-free dome cages) were placed in the base of each arena: one on the left and one on the right. One leaflet was randomly assigned 10 individual TPP, with the other herbivore choice (10 individuals) placed on the other leaflet. The number of prey used was based on a pilot experiment. Once the prey had been placed, a single ladybird was introduced in between the leaflets and left for three hours. The time limit was determined using a preliminary study that indicated that ladybirds took 3 hours for approximately half of all prey to be eaten. Upon the completion of the experiment, the adult ladybirds were euthanized and dissected to determine their sex. An attempt to rear the larval ladybirds to adulthood after the experiment and sex them was unsuccessful because of high mortality rates.

3.3.3 Prey provision

To standardise the size of each prey (Table 3), approximately 60% of TPP provided were 4-5th instar nymphs, 20% 3rd instar, and 20% 1st-2nd instar per arena. Aphid life stages were standardised in a similar way: 60% 4th instar, 20% 3rd instar, 20% 2nd and 1st instar. Whitefly life stages were approximated using a 50/50 mixture of 3rd and 4th instar nymphs. Foxglove aphids were excluded from the experiment due to collection and rearing difficulties.

Table 3. The relative sizes of the prey to the southern ladybird

	TPP	**Green Peach Aphid**	**Whitefly**	**Potato Aphid**	**Southern Ladybird**
Mean (mm)	2.33	1.91	1.23	2.9	7.2
95% CI	0.36	0.35	0.14	0.25	0.39

3.3.4 Analysis

The experiment was designed as a randomised complete block design, with three treatments and 10 replicates. The number of replicates was based on a power analysis from the pilot study. The analysis was done using a one way ANOVA (Genstat 14, VSN International, Hemel Hempstead). The ANOVA compared the mean difference between the percentage of TPP consumed and the percentage of the alternative prey choice consumed between treatments. ANOVA was also used to compare the differences in consumption between TPP

and the other prey choice within treatments. An ANCOVA was used to compare the effect of the covariate of sex.

3.4 Results

There was a significant (df=2 P=0.003) treatment effect of the mean difference between adult ladybirds' percentage of TPP consumed and the percentage of the alternative prey choice consumed in the choice test with adult ladybirds. Using the 5% LSD value, a significant difference was found between the TPP and green peach aphid, and the TPP and whitefly treatments (Fig. 7). A significant difference between the TPP and whitefly, and the TPP and potato aphid treatments was also found (Fig. 7). There was no significant effect of blocking.

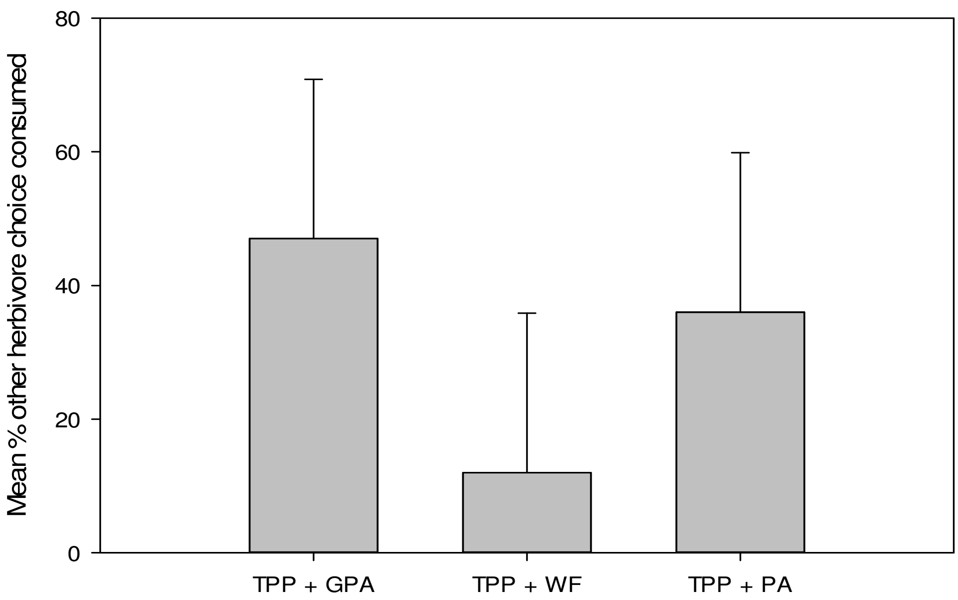


Figure 7. The mean difference in percentage consumption for adult ladybirds using 5% LSD values. GPA is the green peach aphid, WF is whitefly, PA is potato aphid.

There was a significant negative effect on the mean percentage consumption of the alternative prey consumed (df=2 P=0.019). The southern ladybird adults consumed significantly fewer whitefly nymphs compared with green peach or potato aphid nymphs (Fig. 8). There was no significant difference for the mean number of TPP consumed. An ANCOVA to detect differences between the sexes with respect to prey choice for adult ladybirds found no significant differences.

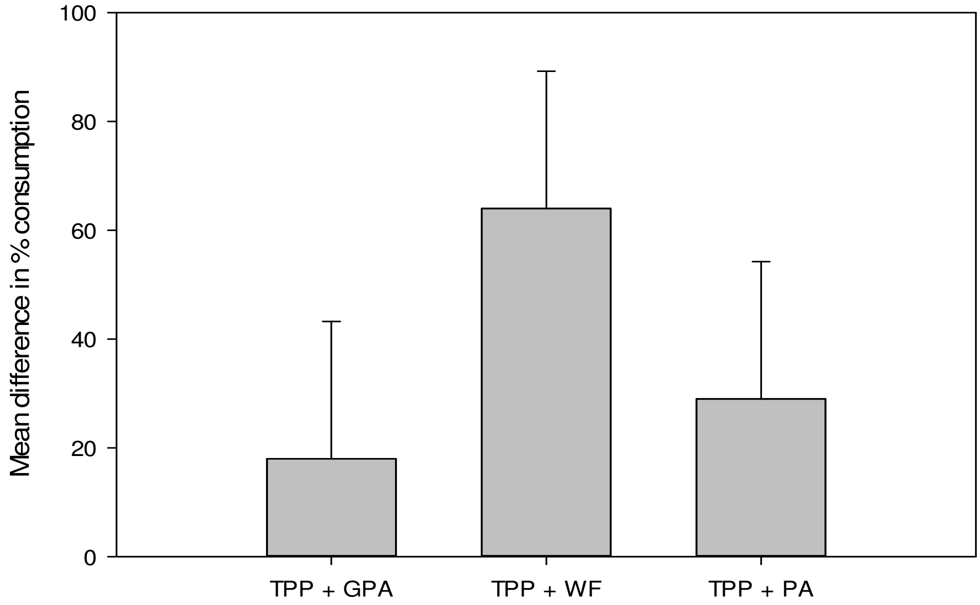


Figure 8. The mean percentage of the alternative prey consumed by adult ladybirds using 5% LSD values. A significantly lower proportion of whitefly was consumed compared to green peach aphid and potato aphid. There was no significant difference in consumption between green peach aphid and potato aphid. GPA is the green peach aphid, WF is whitefly, PA is potato aphid.

The southern ladybird larvae also showed a significant (df=2 P=<0.001) effect on the mean difference between the percentage of TPP consumed and the percentage of alternative prey consumed. Using the 5% LSD value, a significant difference was found between TPP and green peach aphid and TPP and whitefly. Also, a significant difference between TPP and whitefly and TPP and potato aphid (Fig. 9) was found. The TPP + whitefly treatment had a significantly higher mean difference in the percentage consumed than the TPP + green peach aphid and TPP + potato aphid treatments. TPP + green peach aphid and TPP + potato aphid treatments were not significantly different from each other.

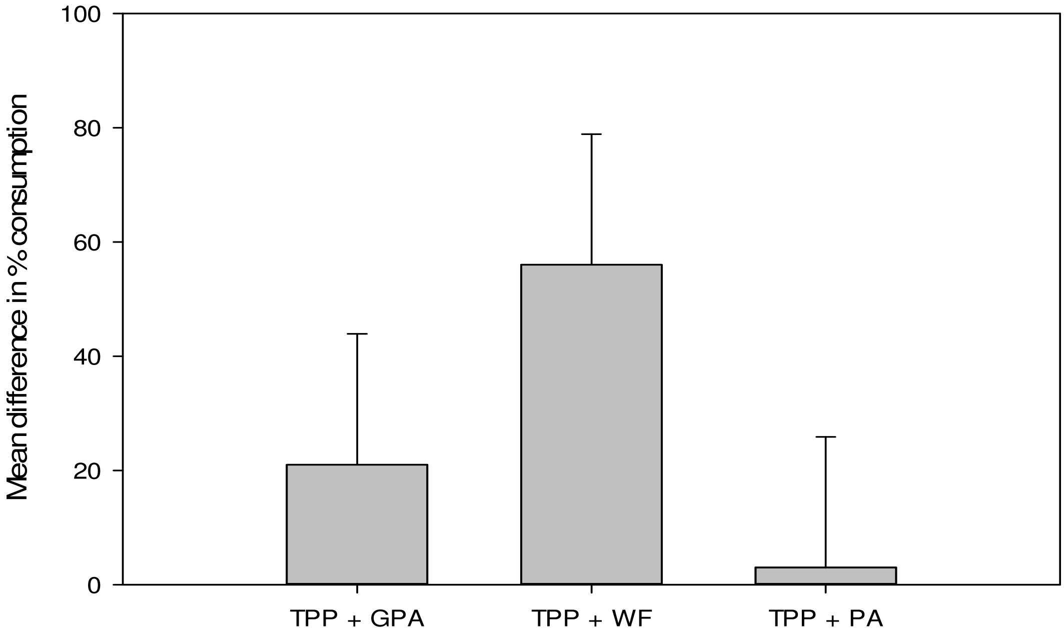


Figure 9. The mean difference in percentage consumption for ladybird larvae with 5% LSD values. GPA is the green peach aphid, WF is whitefly, PA is potato aphid.

There was a significant (df=2 P=0.002) effect on the number of alternative prey consumed. The larvae consumed significantly fewer whitefly than green peach or potato aphid (Fig. 10). There was no significant difference for the mean number of TPP consumed.

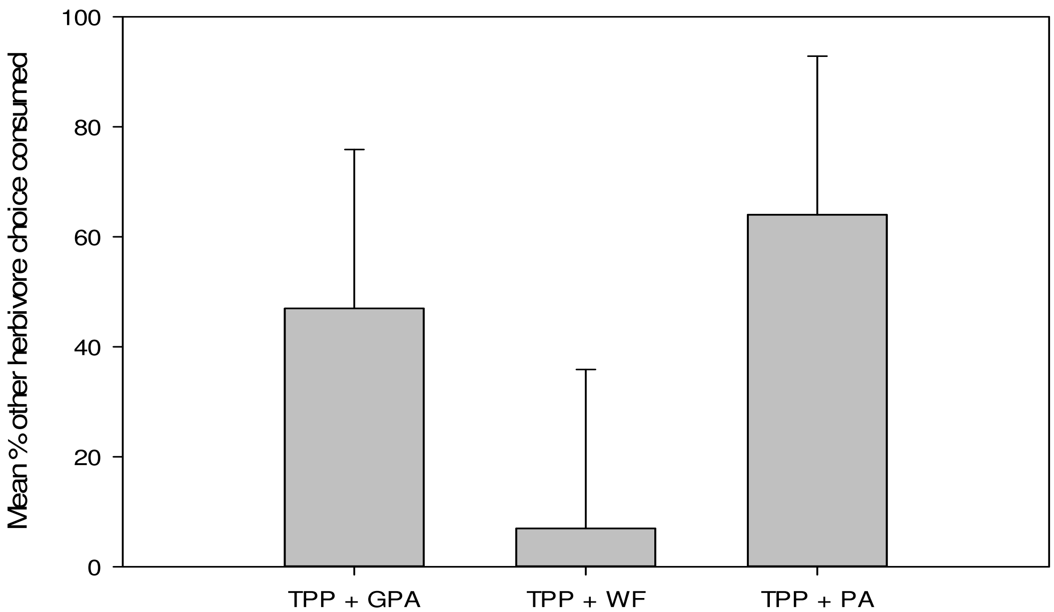


Figure 10. The mean percentage of alternative prey consumed by ladybird larvae with 5% Fishers LSD values. Whitefly was consumed significantly less than green peach aphid or potato aphid. There was no difference between consumption of green peach aphid or potato aphid. GPA is the green peach aphid, WF is whitefly, PA is potato aphid.

3.5 Discussion

This study represents a new species association occurred between the southern ladybird and each of these prey species (see Hokkanen and Pimentel 1984). The results of the choice tests show that ladybird adults and larvae do not preferentially consume TPP nymphs when presented with green peach aphids or potato aphids, as was hypothesised (Figs 7, 9). Ladybirds are often generalist predators throughout their life history, readily consuming a range of prey species (Obrycki and Kring 1998) - southern ladybird is known to be associated with a range of small herbivores in its native habitat (Slipinski 2007). It is uncommon for ladybirds to prey predominantly upon psyllids (Hodek and Honěk 2009), with most studies indicating that psyllid predation is mostly undertaken by aphidophagous or cocccidophagous ladybirds (Hodek and Honěk 2009). The southern ladybird's generalist diet helps explain why no prey preference occurred when presented with small herbivorous potato pests similar to those in its native range and diet.

The significant result for the choice between TPP nymphs and greenhouse whitefly nymphs by ladybird adults and larvae is not due to preferentially predating more TPP, but consuming fewer whitefly (Fig 8, 10). This finding contradicted my hypothesis, possibly because whitefly nymphs and adults usually have an external wax coating formed from lipids (Buckner et al. 1994, Nelson et al. 1994, Buckner et al. 1999, Nelson et al. 2000). It is thought such a lipid coating can provide, among other uses, a deterrent to predators (Gibbs 2002).

Prey capture success is correlated with relative predator body size (Sabelis 1992). This means the southern ladybirds' relative size to the whitefly (Table 3), aphids, and TPP could have lead to the whitefly being overlooked by the ladybirds. Hodek and Honěk (2009) note that biological control of whitefly species by ladybirds is not well explored, but that generalist ladybirds are not very adept at using whitefly as prey, and require more specialised ladybirds to utilise them as prey effectively.

In some instances, psyllids are essential prey items for ladybirds in order to complete their lifecycle (Hodek and Honěk 2009). For example, the Asian citrus psyllid , *Diaphorina citri* Kuwayama is essential prey for the ladybird species *Olla v-nigrum* (Mulsant) (Michaud and Olsen 2004). Bain et al. (1984) found psyllids were necessary to induce oviposition in the southern ladybird when using artificial diets for the purpose of mass rearing. Rearing of southern ladybird by Bioforce Ltd. utilises TPP in the diet to create successful cultures. There is uncertainty if the southern ladybird can complete its lifecycle on a diet of green peach

aphids or potato aphids alone since prey acceptance does not prove it can reproduce using that food source alone (Hodek 1973, Majerus 1994).

Ladybirds are known to regularly accept prey that are not essential for egg maturation, oviposition or for completion of their lifecycle (Hodek 1973, Majerus 1994) commonly utilising alternative prey to ensure survival when preferred prey is scarce (Hodek 1973, Majerus 1994). The starvation period used to standardise ladybird hunger in this experiment may well have led to time-dependant changes in the acceptance threshold of prey, resulting in non-essential or alternative prey being chosen (Browne and Withers 2002). Coccinellid larvae are reported to increase their dietary range and increase their ability to digest a more varied diet in relation to increasing larval instar stages (Lundgren and Weber 2010), suggesting that earlier instar larvae may be more selective in their prey choice.

The present study's findings clearly demonstrate that the southern ladybird does not have a prey preference when presented with the trialled pest species. However, the use of small experimental arenas may have affected the findings. Withers and Browne (2004) suggest that small experimental arenas disrupt the usual sequence of prey-finding behaviours, which can lead to a wider range of host acceptance than is realised in the field (Withers and Browne 2004). By removing the cues that are detected at distance, the natural enemy relies on the physical contact cues with the prospective prey to be used to determine if the prey is suitable (Withers and Browne 2004). By interrupting the prey detection sequence, this means a natural enemy might accept a prey item that it would not respond to at a larger distance (Withers and Browne 2004). Therefore in a true crop setting, the southern ladybird may not respond to cues from TPP, green peach aphid, potato aphid, or whitefly. Based on the close association between the southern ladybird and similar prey in its native range, one could expect the ladybird to respond to cues from such prey in the field.

A study by Stufkens and Teulon (2001) found that most aphids in potatoes occurred on the lower leaves of the plant, with few found on the upper and middle leaves. Sampling of TPP nymphs has found that they predominantly occur on the underside of middle and upper leaflets of potato plants (Butler and Trumble 2012b, Martini et al. 2012). Spatially, where a ladybird feeds on a crop is variable with some species preferring lower parts of the plant, and others the middle and/or top leaves (Hodek 1973). The implication may be that the southern ladybird prefer to forage on a part of the plant where TPP is more likely to be present than aphids.

3.5.1 Conclusions and future research

When either green peach aphid or potato aphid was presented as an alternative, no prey preference was detected. However, this lack-of-preference is not necessarily an undesirable finding. These two aphid species are major pests of potatoes, so additional predation pressure could be considered beneficial, presuming other minor pests are not released from competitive exclusion or if intraguild predation reduces prey mortality. The southern ladybird may have potential to complement other generalist predators already present in potato crops.

The southern ladybird as both adults and larvae showed an aversion to consuming whitefly when TPP was also present. Aversion by the southern ladybird to accepting whitefly as a prey item can also be viewed favourably. The opportunity is therefore created that the southern ladybird complements existing IPM in glasshouse-grown tomatoes, where it potentially could consume TPP and not interfere with established parasitoid biological control of whitefly. Further study in the context of tomatoes would be needed to elucidate and further the results found in this study.

Further work is required to determine if the southern ladybird has a spatial preference at the plant scale to determine if it has a preference foraging in the lower, middle, or upper parts of the host plant, as this could influence its probability of encountering and consuming TPP in the field.

The results of this study could be verified and furthered by testing the prey preferences of the southern ladybird when presented with the same prey choices at different ratios to one another. Different prey ratios can influence the choice of the predator and so can be an important step in assessing prey preference. Also, varying the ladybird starvation times could elucidate if less hungry ladybirds are more discerning in their prey choice.

Future research could examine if there is a prey preference testing against native psyllids or aphids.

Chapter 4
Southern ladybird longevity on low prey availability and a floral resource

4.1 Abstract

Enhancing biological control agents to improve pest control is an important area of research in pest management. Increasing the southern ladybird's potential as a biological control agent of TPP is critical. This chapter examines how a floral resource and low prey provision affect the longevity of the southern ladybird. Buckwheat, TPP, and buckwheat + TPP combined all significantly increased the southern ladybird's longevity compared to starved ladybirds. Ladybirds fed the combination of buckwheat and TPP lived significantly longer than those only fed TPP. This chapter shows that the southern ladybird is capable of surviving for a biologically useful period, from a biocontrol perspective, on a diet of low prey abundance, and that a source of buckwheat has potential as a useful tool for increasing southern ladybird longevity when prey is scarce, such as in early-season conditions.

4.2 Introduction

Conservation biological control is the practice of enhancing natural enemy populations and efficiency by engineering the habitat to provide access to food resources, alternative prey or hosts, or shelter from unfavourable environmental conditions (Landis et al. 2000, Landis et al. 2005, Zehnder et al. 2007). This form of pest control has been widely demonstrated to have potential for supporting crop pest control actions (Jonsson et al. 2008), theoretically reducing agrichemical inputs by keeping pest numbers below economic damage thresholds (Gurr and Wratten 2000). One conservation biological control method is to provide floral resources such as buckwheat as a diet supplement.

Coccinellids have been utilised as a part of classical biological control programs, often as an aphid predator (Obrycki and Kring 1998, Obrycki et al. 2009). However, they remain relatively underutilised in conservation biological control programs despite considerable scope for increased use of ladybirds in this context (Obrycki and Kring 1998, Obrycki et al. 2009).

Coccinellids adults, more frequently than larvae, use alternative foods in their diets (Majerus 1994, Coll and Guershon 2002, Wäckers et al. 2008, Lundgren 2009). Non-prey foods encompass a wide array of resources, including: nectar, pollen, plant sap, vegetation, fungus, fruit, insect eggs, honeydew and scavenged arthropods (Hodek 1973, Majerus 1994, Lundgren 2009). The value of alternative foods is primarily in survival of individuals when prey is scarce (Hodek 1973, Majerus 1994), but also when increasing or replacing lipid and glycogen reserves before or after dormancy (Majerus 1994). Access to such resources may be critical in enabling ladybirds to suppress early-season pest densities and reduce later outbreak levels by ensuring their survival when prey is scarce (Obrycki et al. 2009). Clearly, it is important that these dietary requirements be taken into account in conservation biological control programmes using coccinellids (Lundgren 2009).

Some coccinellids are true omnivores (Coll and Guershon 2002); for these species access to plant resources such as nectar and pollen is essential to maintain biological control. Access to floral resources can influence predatory biological control on two temporal scales, the day to day consumption of pests, and the longer term effects on predator longevity and fecundity (Robinson et al. 2008). Over the short term, the presence of alternative food may reduce per capita consumption of target prey by omnivores (Eubanks and Styrsky 2005, Robinson et al. 2008), but potentially can increase ladybird longevity and fecundity causing greater mortality of the pest population in the long term (Pemberton and Vandenberg 1993).

Whether the southern ladybird is a life-history omnivore (single life stage) or a true omnivore (consecutive life-stages) can have an important impact on potential prey density reductions – a single life-stage omnivore will utilise floral resources to survive at low prey densities (Jonsson et al. 2009), while a true omnivore may more readily consume floral resources, reducing prey predation levels (Robinson et al. 2008).

Floral resources and extrafloral nectar have been extensively documented as being used by ladybirds in natural and agricultural settings (Hodek 1973, Pemberton and Vandenberg 1993), with both nectar and pollen considered critical to their diet (Hodek 1973). Experimental data which demonstrates ladybird longevity or fecundity being affected by the provision of floral resources in the field is lacking (Obrycki et al. 2009), even laboratory studies have failed to quantify how floral or extrafloral nectar affects ladybird longevity (see Pemberton and Vandenberg 1993, Bertolaccini et al. 2008, Obrycki et al. 2009). However, it should be noted that floral nectar and extra floral nectar differ in their sugar composition, particularly types of sugar with floral nectar also often containing additional defensive secondary metabolites

(Wäckers 2005, Lundgren 2009). Conversely, research has demonstrated that a diet composed exclusively of pollen, without access to prey, can be detrimental to the developmental and reproductive life-history traits of ladybirds (Berkvens et al. 2008).

Buckwheat has been widely researched and used in conservation biological control efforts as a floral resource in agricultural and horticultural systems (Landis et al. 2000, Robinson et al. 2008, Jonsson et al. 2009). Buckwheat has been found to increase natural enemy numbers where it is present (Stephens et al. 1998), increase parasitism rates (Stephens et al. 1998, Berndt et al. 2002) and extend longevity (Irvin et al. 2006, Robinson et al. 2008). Even access to artificially supplied sucrose has been found to retain the southern ladybird relatively aggregated in the target area of a eucalyptus plantation, and improve its efficacy as a biological control agent (Mensah and Madden 1994). As mentioned above, there are no known published studies on factors influencing the longevity of the southern ladybird.

This chapter aims to determine the effect of a floral resource and low prey provision, imitating early season conditions, on the longevity and potential fecundity of adult southern ladybirds. Buckwheat was used as the floral resource, with TPP as the prey. Longevity was recorded daily, and all ladybirds were dissected upon death to determine sex and were stained with anthrone reagent to determine nectar or pollen consumption. Although this study measured ladybird survivability, rather than longevity *per se*, the two terms are used interchangeably in the literature (e.g., Lavandero et al. 2006, Robinson et al. 2008).

4.3 Methods

4.3.1 Experimental design

The study was conducted using newly-emerged (<4 days) southern ladybirds (BioForce Ltd, Auckland). The ladybirds were provided with a mixture of excess TPP and green peach aphids prior to the beginning of the experiment. The experiment comprised three treatments and a control: 1) water only, 2) buckwheat only, 3) TPP only, 4) TPP + buckwheat. Water was provided in every treatment and consisted of a 1.5 ml microcentrifuge tube filled with water and a moist cotton roll in the top. This was attached to the inside of each experimental container and replaced every three days. One ladybird, of unknown sex, was placed into each experimental container.

A bamboo frame with an individual upright pole for each container was used (Fig.10). This enabled the containers with buckwheat treatments to be vertically adjusted depending on the height of the most apical buckwheat (cv. Katowase) inflorescences. Each experimental

container measured 18.5 cm by 10.5 cm and had a foam plug in the bottom with a slit to allow for a buckwheat stem. This enabled containers to be placed over buckwheat inflorescences rather than providing excised buckwheat. There was a small foam plug in the side to allow TPP to be added. The most apical inflorescence was used on each buckwheat plant, and any leaves that ended up in the containers removed. The buckwheat was replaced weekly to ensure a constant supply of nectar and pollen. The ladybirds were checked daily and 10 TPP added to the appropriate experimental containers. The TPP were provided on a piece of potato (cv. Desiree) leaflet placed in a 3 cm plastic dish placed at the bottom of the experimental container. The TPP provided were an approximate mix of 1/3 each of nymph instars 3, 4, and 5. The potato leaflet used on the previous day was removed daily.



Figure 11. The frame set up for the longevity trial in the temperature control room.

The experiment was undertaken in a temperature controlled room at 24°C ±2°C, and a 16:8 light to dark ratio for 12 weeks. When a ladybird was recorded as dead, it was removed, frozen, and later dissected to determine its sex and stained with anthrone reagent. Anthrone reagent was used following the procedure used by Robinson et al. (2008) to determine if ladybirds had consumed buckwheat nectar, pollen, or both.

4.3.2 Analysis

The experiment was designed as a 2x2 factorial in a randomised complete block design, three treatments and a control, with eight replicates. The analysis was performed in Genstat 14 and

SPSS 19 using a one-way ANOVA with 5% LSD for multiple comparisons between treatments. Kaplan-Meier analysis was done using Genstat 14.

4.4 Results

There was a significant treatment effect (df=3, P=<0.0001), but no effect of blocking. Using a multiple comparison test (LSD 5%), as expected, all treatments with food resource provision were significantly longer in longevity from the control (Fig. 12). Treatment two (buckwheat only) was significant at P=0.002, treatment three (TPP) at P=0.009, and treatment 4 (buckwheat + TPP) P=<0.0001. The treatments of buckwheat only and TPP only, and buckwheat only and buckwheat + TPP were not significantly different from one another. However, TPP only and buckwheat + TPP were significantly different to each other (P=0.025) indicating that the ladybirds with access to buckwheat flowers and TPP lived longer than ladybirds with access to only TPP. There was no effect of ladybird sex on survival rates.

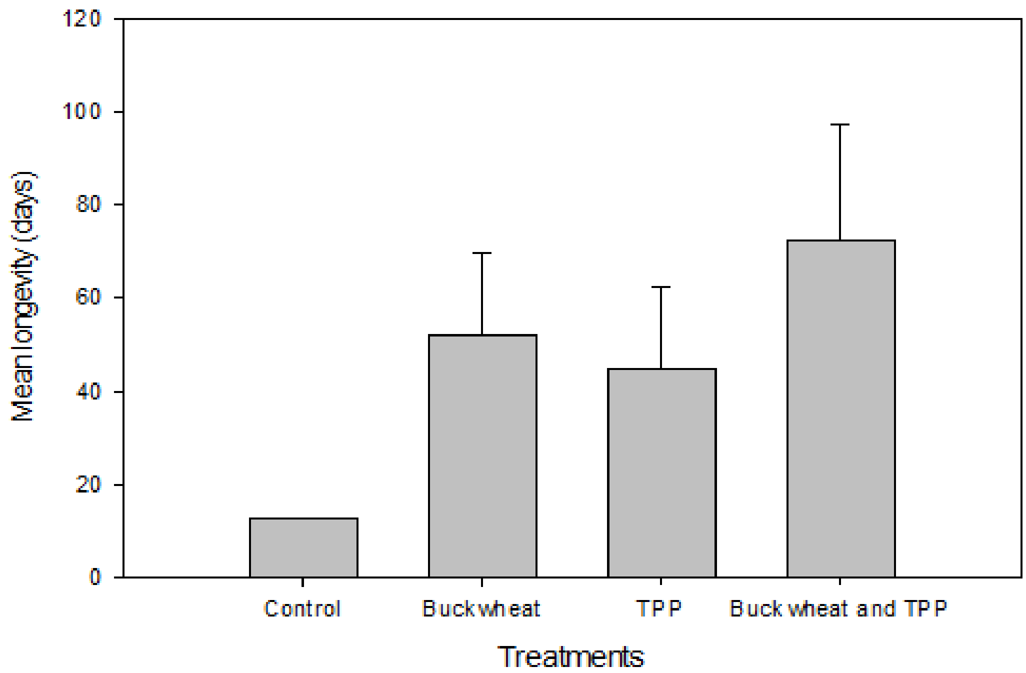


Figure 12. The mean longevity of the adult southern ladybird in each treatment, shown with LSD 5% error bars.

None of the control ladybirds lived for the 12 week duration of the trial. Of the ladybirds with only buckwheat, 37.5% of the ladybirds survived throughout, while only 12.5% (a single ladybird) survived the trial duration with only TPP. 62.5% of the ladybirds provisioned with buckwheat and TPP survived the trial duration.

A Kaplan-Meier analysis (Fig. 13) illustrates the effect of the different treatments. The survivorship of the ladybirds in the control treatment rapidly declines, with 100% mortality by day 14. Treatment two showed a steady decline in ladybird survivorship up to days 20-30 before plateauing until the end of the experiment. Ladybirds in treatment three showed a rapid decline in the probability of surviving between days 30 and 40, and continued their steady decline until the termination of the experiment. The survivorship of ladybirds in treatment four declines gradually from the start to end of the experiment.

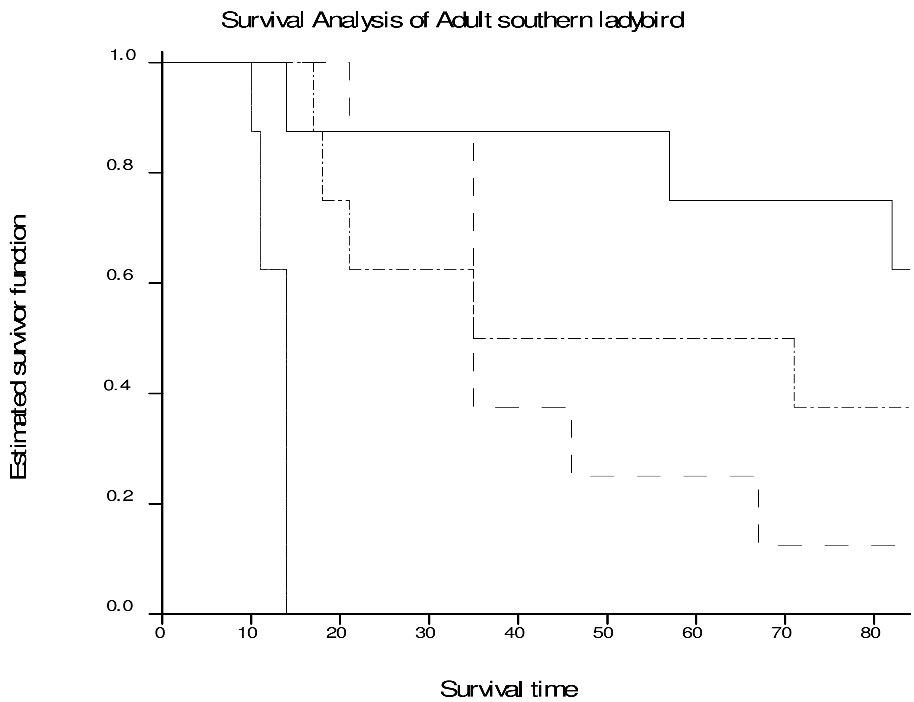


Figure 13. Kaplan-Meier analysis showing the estimated probability of survival for individuals in each treatment. Treatment one dotted line (….), treatment two dot and dash (-·-·-), treatment three dash (- - -), treatment four solid(—).

All ladybirds were dissected and their gut contents stained with anthrone reagent. As expected, none of the control ladybirds, or those in treatment three tested positive for either buckwheat nectar or pollen. Of the ladybirds in treatments two and four, 100% tested positive for buckwheat nectar, and 31% for buckwheat pollen. In treatment two, 2 ladybirds (25%) tested positive for pollen and in treatment four, 3 ladybirds (37.5%) tested pollen positive. There was no difference in pollen consumption between the sexes.

4.5 Discussion

Unsurprisingly, the ladybirds in the unfed control treatment had high mortality. The longest any survived in this treatment was 14 days. However, under the lower temperatures in natural

conditions one might expect ladybirds to live longer The higher a temperature is, the shorter the longevity (Pervez and Omkar 2004, Mori et al. 2005, Aksit et al. 2007), probably due to higher temperatures causing higher metabolic rates, leading to a more rapid use of energy and increasing the rate of aging (Dixon 2000).

No other studies using floral nectar resources, as opposed to extra-floral nectar resources, and its effects on ladybird longevity could be found. That buckwheat increased the longevity of the southern ladybird is consistent with other generalist predator and ladybird studies. For example, Lundgren and Seagraves (2011) compared the provision of extra-floral nectar to the ladybird *Coleomegilla maculate* De Geer, 10 days before access to high quality prey, while others had access to prey only. Survival of the ladybirds increased by 50% when they had access to extra-floral nectar compared with those that did not. Robinson et al. (2008), investigated the effect of buckwheat on the longevity of the brown lacewing (*Micromus tasmaniae*) and found in a laboratory bioassay that buckwheat nectar and pollen significantly increased this predator's longevity relative to the starved control by a minimum of 17 days.

Psyllids are considered essential prey for the southern ladybird (Bain et al. 1984). In the current experiment, ladybirds feeding on only TPP showed a shorter longevity compared to those ladybirds provided with TPP and buckwheat. This is most likely the result of providing only 10 TPP daily, simulating early season TPP densities. The southern ladybird can consume up to 100 TPP in 24 hours, as demonstrated in a bioassay by O'Connell et al. (2012). The combination of essential prey with a floral nectar resource equates to greater food availability and therefore lower likelihood of starvation relative to a diet of only TPP. A seperate experiment by Robinson et al. (2008), also with brown lacewings did not find a difference between prey only, and buckwheat and prey treatments. This is most likely because the experiment was terminated prematurely after just 20 days, with most fed lacewings still alive.

Pollen is amongst the most nutritious non-prey foods a ladybird can consume, and its place as a component in natural enemy diets is a well-studied subject (Samways et al. 1997, Triltsch 1997, Lundgren 2009). Ladybirds are known to consume anemophilous (wind dispersed) pollen, not zoophilous (animal dispersed) pollen (Lundgren 2009) such as that of buckwheat, except where zoophilous pollen is provided in artificial diets (e.g., Bonte et al. 2010). Demonstrating that the southern ladybird will indeed consume buckwheat pollen is an important finding and warrants further investigation, particularly if ingestion changes over time. It is plausible that the reason any pollen was consumed at all in this experiment was by accidental ingestion. During the experiment the ladybirds were observed feeding on nectar in

the plant, and in the process the head and thorax became coated in pollen, later grooming may have led to the consumption by the five ladybirds which tested positive. This accidental ingestion could be nutritionally important.

The ladybirds used in this trial were unmated. Data presented by Dixon (2000) shows that unmated female and male ladybirds live longer than mated counterparts. However, this data did not account for how factors such as number of mating's can influence longevity. Therefore, it could be expected that the southern ladybird, when mated, may not live as long as this study indicates. Mishra (2005) found in two of their three species studied (*Cheilomenes sexmaculata* Fabricius and *Propylea dissecta* Mulsant), that increased number of mating events lead to reduced longevity for both males and females. It is proposed that this phenomenon is caused by the energy costs associated with courtship and mating, egg production, and egg laying. However, a similar study using *Adalia bipunctata* L. found no effect on longevity by mating (Perry and Rowe 2008).

4.5.1 Conclusions and Future Research

The research here demonstrates that buckwheat is a useful floral resource for the southern ladybird which can significantly extend longevity. This easy-to-grow plant can be easily incorporated into potato fields, even if only on the margins where TPP densities are highest. Buckwheat could form an important food source in early-season conditions when TPP numbers are low, helping ensure the southern ladybird's survival if released into a field. That the southern ladybird can survive a relatively long time on just 10% of the recorded maximum daily consumption of TPP is encouraging, and research into whether it can reproduce with low TPP provision is an important next step. Also, whether this predator is capable of reproducing with access to only buckwheat floral resources, and buckwheat with low TPP provision should be investigated. It would also be useful to test utilisation of buckwheat floral resources with and without pollen to determine what, if any effect the pollen has on longevity and/or fecundity.

How these results translate into potential impacts on predator-prey dynamics warrants further investigation. Buckwheat could be an important resource in potato monocultures for the southern ladybird, particularly in early season conditions when TPP and other potential prey are scarce. When prey are scare, floral resources can help decouple the relationship between predator and prey, enabling the predator to provide greater top-down control of the pest (Polis and Strong 1996, Eubanks and Styrsky 2005). However when prey, TPP in particular, are more abundant and when high prey consumption is vital to regulate the pest population,

buckwheat nectar consumption could be at the expense of per capita prey consumption (Eubanks and Styrsky 2005). Importantly, Spellman (2006) found that buckwheat did not reduce consumption by the ladybird *H. axyridis* Pallas of the aphid *Aphis spiraecola* when presented with 20-50 individuals.

When the southern ladybird is released into a crop, access to buckwheat floral resources may help prevent migration away from the crop caused by low prey numbers. Other non-crop flowers utilised in conservation biological control techniques, such as the commonly researched phacelia (*Phacelia tanacetifolia* (Borginaceae)), and sweet alyssum (*Lobularia maritima* (Brassicaceae)) are also worthy of investigation in regards to their impact on the survival and potential longevity of the southern ladybird.

Chapter 5
Conclusion

5.1 Conclusions and future research

The overall aim of this thesis was to assess key aspects of the southern ladybird's ecology to provide insight into its merit as a biological control agent for TPP. In the process of investigation the following objectives were achieved:

1. The southern ladybird's ability to reduce TPP densities on potato plants and whether that led to a trophic cascade from top-down control was investigated
2. Southern ladybird prey preferences between TPP and common small herbivorous prey on potatoes was assessed
3. The longevity of the southern ladybird with low prey provision, access to floral resources, or a combination of both was determined.

Objective 1: Key glasshouse results, implications, and future research

The results are highly encouraging, in that the southern ladybird reduced TPP densities, even with a high TPP inoculation quantity. While the number of tubers and their fresh weight was significantly reduced where the southern ladybird acted as a predator on TPP compared to no TPP and ladybird only treatments, no tubers formed in the TPP only treatment. This result strongly suggests that the southern ladybird was able to exert some top-down control on TPP populations, indirectly influencing plant production.

The inability to show a clear foraging preference by the southern ladybird for any particular part of the potato plant may be due to the need for more appropriate experimental design or more thorough observation during the study. Differences in plant architecture can influence predator foraging strategies and prey predation (Kareiva and Sahakian 1990, Clark and Messina 2003). Despite the southern ladybird not showing a preference for where TPP nymphs occur in the highest densities on an intra-plant-distribution scale (the middle and upper areas), a significant reduction in TPP densities was still found.

Future trials could experiment with fewer TPP being used to inoculate the potato plants, and efforts made to sex the adult TPP before release to help standardise herbivore pressure. Because the TPP used in this experiment (chapter 2) were unsexed, some replicates may have received more females, and therefore, potentially, more TPP over time. Sexing the TPP would enable greater control over this potential variability. The proof of concept in the achievement

of a reduction in TPP densities and a trophic cascade show the southern ladybird to have potential as a biological control agent. Additional experiments could include manipulative field cage trials to validate these results under semi field conditions or to investigate combinations of natural enemies. Alternatively, small scale field releases could be tried. Other contexts should be considered too, such as glasshouse crops infested by TPP.

The results of the glasshouse trial must be considered within the context and setting in which the experiment was conducted. In other words, it comprised a simplified system which had reduced abiotic variation compared with a regular potato crop. Therefore, the results should not be extrapolated to what may occur in a field setting.

Objective 2: Choice test key results, implications, and future research

This study showed that the southern ladybird adults and larvae did not have a prey preference when presented with a choice of TPP and either green peach aphid or potato aphid. It also demonstrated that ladybird adults and larvae had an aversion to consuming whitefly when also offered TPP as a prey choice.

The absence of the southern ladybird having a prey preference when presented with aphids as a choice is not necessarily a negative result. The aphid species used in this experiment are themselves major pests. Any additional predation pressure on this suite of pests is potentially beneficial to potato growers, but may result in less predation pressure on TPP, the target pest.

The relative avoidance of consuming whitefly may also prove important in any future work in a glasshouse context where whitefly is a significant pest, sometimes managed using parasitoids. If the southern ladybird had been found to readily consume whitefly, then that could readily have led to interference with the parasitoid biological control. Even if a prey preference for TPP had been found, this does not necessarily mean there will be a regulatory effect by the southern ladybird.

It is important to realise that this experiment does not measure prey choice directly. The number of a particular prey remaining after the experiment are used as a proxy to indirectly measure prey choice and as such, to truly determine prey choice, the experiment should be repeated using a y-tube apparatus. In that form of choice testing, the direction the ladybird chooses in relation to the volatiles emitted from two different prey choices is indicative of prey choice. Alternatively, providing prey choice in small experimental arenas with the two prey at different ratios can also be important in determining prey preferences.

Objective 3: Longevity key results, implications, and future research

Southern ladybird longevity was not formally quantified until this study (chapter 4). The treatments of buckwheat and low prey provision both significantly extended the longevity of the adult southern ladybird in comparison to the unfed ladybird control. The treatment of low prey provision + buckwheat extended ladybird longevity the most, where the ladybirds in this treatment lived significantly longer than in the low prey provision treatment and control.

If the southern ladybird were to be released into a crop, this research implies that the adults could survive for an average of 45 days with low prey availability. However, if buckwheat were to be supplied as a floral resource, even in the absence of any prey (such as very early season), the buckwheat nectar is likely a sufficient resource for survival (Fig. 12) before prey arrive and colonise the crop. When both prey and buckwheat were available, the ladybirds lived for an average of 72 days. Nearly two thirds survived the 12 week experimental duration – This parallels a large proportion of the growing time for most potato cultivars growing in New Zealand.

The enclosed nature of this experiment and the controlled environment in which it occurred may mean the longevity found here could be different to that realised in the field. Longevity differences could be due to increased energy expenditure searching for prey or other foods, and abiotic variability effecting metabolic processes.

Ladybirds are synovigenic (Dixon 2000) meaning that females mature eggs through their lifespan. This makes access to suitable food critical in determining total lifetime fecundity (Dixon 2000). How only low prey numbers, access to buckwheat, or both affects ladybird fecundity requires further investigation as this has implications for overall predation pressure by the southern ladybird on prey. If low prey, buckwheat, or both results in low or reduced fecundity, this has implications for the ladybird forming self-sustaining populations after a release.

5.2 Considerations for a field release

The research in this thesis has demonstrated that buckwheat as a floral resource can increase the longevity of the southern ladybird (chapter 4). However, previous research in other cropping contexts has demonstrated that buckwheat can increase the longevity and oviposition of a pest, potato tuber moth (*Phthorimaea operculella* Zeller). Baggen and Gurr (1998) found that intact buckwheat flowers, honey, and coriander (*Coriandrum sativum* Apiaceae) flowers

increased the potato tuber moth's longevity and oviposition. Neither nasturtium (*Tropaeolum majus* Tropaeolaceae) nor borage (*Borago officinalis* Boraginaceae) had an effect on the moths' longevity or oviposition.

Based on these results, where potato tuber moth is a problem, buckwheat should be used with caution as a floral resource, as both ladybird and moths' may benefit. Alternative floral resources for the southern ladybird that does not provide an advantage to the potato tuber moth, need to be investigated.

Beyond basic manipulative experiments, could crop losses either through reduced planting space (by using space normally planted with crop for the floral resource) or competition with a floral resource lead to reduced crop yield overall, or does potential increased biological control mediated by the floral resource more than compensate for such losses? This is an important question to answer if this biological control is to have real world use for growers.

Other natural enemies that consume TPP occur in potato crops nationwide (Jorgensen et al. 2011, Walker et al. 2011) (chapter 1). Thus, releasing the southern ladybird into a crop infested with TPP could be considered attempting to have an additive effect. This is a tactic utilised in many classical biological control programs around the world for insects, although is not best practise (Denoth et al. 2002). The successful establishment of biological control agents, when using multiple to control an insect pest is lower than that of only using/releasing a single biological agent (Denoth et al. 2002).

The problem with using multiple biological control insects is that simply adding another biological control agent, such as the southern ladybird, may not have any effect on TPP, and its establishment may be influenced by the natural enemies already present (see chapter 1). For example, intraguild predation is common amongst insect biological control agents (Rosenheim et al. 1995, Symondson et al. 2002) and this interaction may reduce the likelihood of southern ladybird establishment and or its effectiveness.

A concept frequently examined when intercropping two or more different crops is how yields or quality are affected by the competition between the two or more crops (Willey and Rao 1980, Baumann et al. 2002). However, how competition might apply to the planting of floral resources among a crop is a concept that has yet to be thoroughly explored. How any floral resource may affect yields at the entire crop level remains unclear. This topic has been the subject of a pilot study, investigating the effect of buckwheat on pak choi fitness as measured by seed yields (Dean O'Connell, pers. comm., Lincoln University 2013). Buckwheat has

increased seed yield in the presence of pak choi, but there did not appear to be any effect on pak choi seed yield.

5.3 Future research

Releasing the southern ladybird into a crop is not completely without risk. The southern ladybird could prey on other natural enemies. It is therefore critical to undertake intraguild predation trials. Intraguild predation is where organisms in the same trophic level share a prey and may also prey upon or parasitise each other (Rosenheim et al. 1995). This intraguild predation has been frequently shown to disrupt biological control and associated trophic cascades in arthropods (Rosenheim et al. 1995, Costamagna et al. 2007). The intensity of any intraguild predation would influence any future field releases of the southern ladybird.

If the southern ladybird is released into a field setting, it would be useful to know exactly what it eats. Therefore molecular tools could be useful to determine the prey consumed via PCR based analysis of the ladybird gut contents (e.g., Thomas et al. 2012). This method could also be a potentially useful method to detect intraguild predation by the southern ladybird.

Another basic question that needs an answer is whether the southern ladybird consumes all life stages of TPP. Although observations have been made that it will indeed consume adult TPP in small experimental arenas (Catherine Hosted, unpublished data), it is unknown whether eggs would be consumed. A simple no-choice test with TPP eggs laid on a potato leaflet could readily confirm this.

With the caveat of interim research being required, well designed, large scale field releases of the southern ladybird around the country, at multiple sites in each region, would enable a comprehensive and thorough evidence-based conclusion as to whether the southern ladybird works as a biological control agent of TPP at multiple temporal and spatial scales.

A primary concern about field releases of the southern ladybird is that it will rapidly disperse out of the release site crop. It is also important to consider other settings in which the southern ladybird could be utilised. Glasshouse crops such as capsicums or tomatoes could help remove this issue by providing an enclosed area, reducing the chances of the ladybird dispersing.

5.4 Final Summary

The southern ladybird shows some potential as a biological control agent for the invasive tomato-potato psyllid, and thus may provide another 'tool in the toolbox' for the management of TPP in potato or other solanaceous crops. This research has provided proof of concept of the potential for reducing TPP densities on potato plants, leading to a trophic cascade of increased tuber numbers. This ladybird did not demonstrate any particular preference for any of the common small herbivore prey which occurs in potato crops, but as a generalist predator, this is not unexpected. The longevity of the adult ladybirds depended on the food provided. The ladybirds provided with buckwheat or TPP + buckwheat survived the longest - those provided with only TPP survived longer than the unfed control, but significantly shorter than ladybirds supplied with buckwheat. Further research is required before a full-scale field release of the ladybird. Only such large scale trials and releases will determine if the southern ladybird is an effective biological control agent of TPP.

Appendix A.

A note on the glasshouse breeding of the southern ladybird (*Cleobora mellyi*)

An attempt was made to determine if the southern ladybird could complete its lifecycle on a diet of just TPP as it is commercially reared on a mixed diet consisting of other small herbivores such as aphids. Suggested artificial diets also exist (Bain et al. 1984), but relying on such diets can lead to a reduced ability to attack real prey once released (Dixon 2000).

Adult TPP were released on a potted potato plant in a dome cage as in chapter two methods. The TPP were allowed enough time to complete approximately two generations. At this stage, seven unmated adult southern ladybirds' of unknown sex were released into the single dome cage. These ladybirds were individually marked using queen bee numbering dots. The culture was checked several times a week, but not at regular intervals.

It was not until week two that the first eggs were seen in the cage, although mating had been observed the week prior. Two patches of the ladybirds yellow eggs were laid, and by the following week, most had hatched. Cannibalism rates appeared to be low until the larvae reached approximately 4th instar, when their numbers greatly reduced, and parts of larval exoskeletons, such as abdomens and parts of the thorax with some legs were found. Why cannibalism occurred mostly at this instar is unclear as there was ample TPP prey still available. The larvae mostly pupated on the side of the dome cage (Fig. 13), rather than on the plant, and emerged after approximately 4-7 days. Within four weeks, a generation had been completed, with all the founding adults still alive.

Approximately three weeks later another generation was completed, meaning three overlapping generations were in the cage together. This resulted in higher levels of cannibalism of the new larvae and eggs laid. Even some ladybirds pupating were observed to be attacked, despite a high number of TPP still on the potato plant.

The observations indicate that the southern ladybird can complete multiple generations on a diet exclusively composed of TPP. Further work to compare how fecundity, hatching success and longevity of the ladybird is potentially influenced by a diet of only TPP versus the psyllids and other prey it naturally encounters in its native range of Australia is worthy of

investigation. How a diet of just TPP versus a mixed diet of TPP and aphids, and artificial diets should also be investigated to determine the most cost efficient diet.

The observations made here also highlight the importance of reducing cannibalism in trying to keep the southern ladybird in culture. Steps such as removing adults to a separate colony after egg laying and providing excess diet that is easily accessible is likely to be critical to this. Moving larvae, particularly the later stages into separate containers is also advisable at the greatest rate of cannibalism appears to happen at approximately the fourth instar stage.

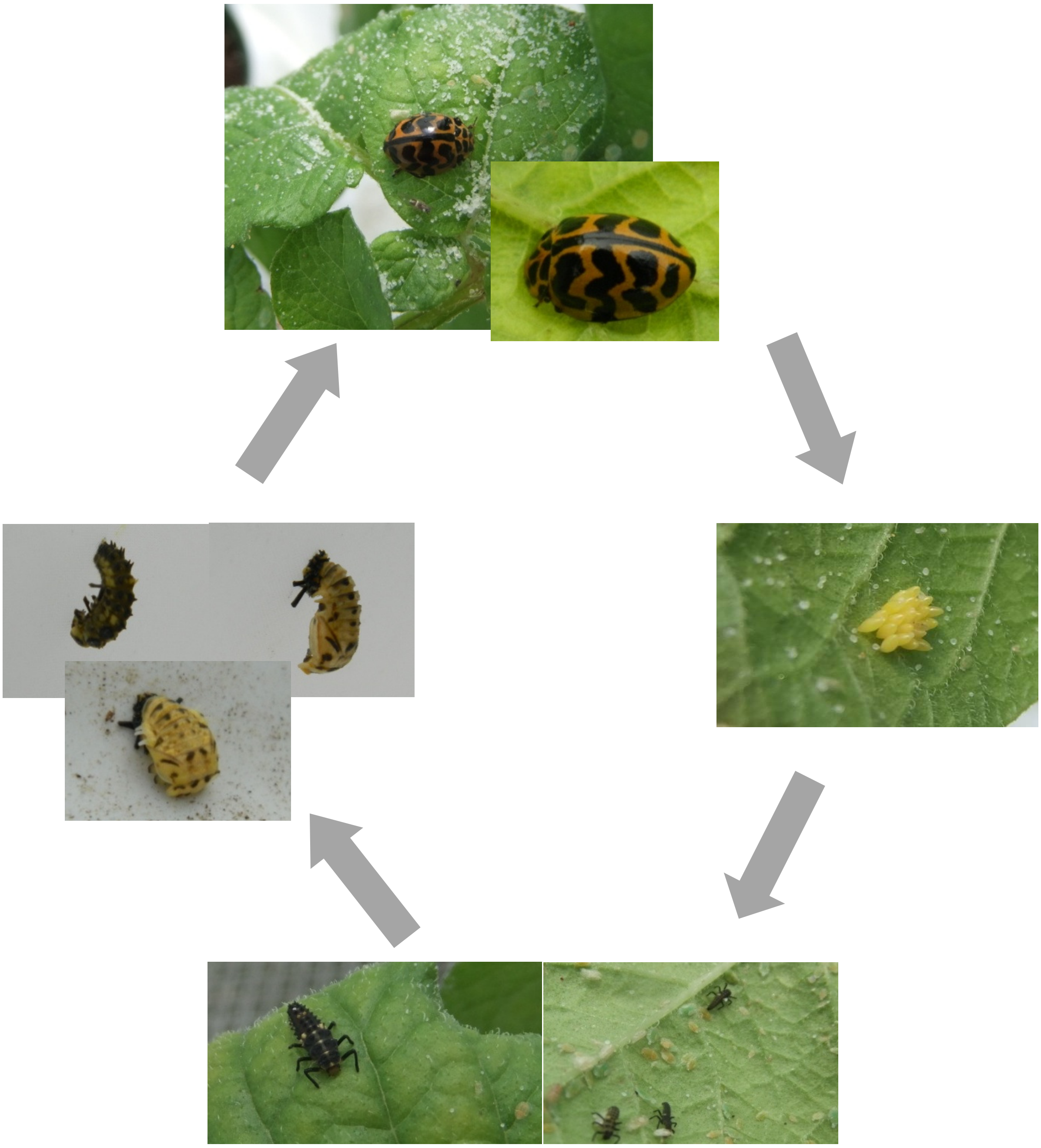


Figure 14. Pictorial summary of the lifecycle of the southern ladybird on potatoes.

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